MULTIELEMENT ANALYSIS IN WHOLE BLOOD USING A CAPACITIVELY COUPLED MICROWAVE PLASMA ATOMIC EMISSION SPECTROMETER

By

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I would like to dedicate this dissertation to my parents, Arthur and Audrey Besteman. Their love and support has meant so much to me. I am very blessed to have them as my parents.

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A capacitively coupled microwave plasma atomic emission spectrometer (CMP-AES) has been evaluated as a clinical method for the direct analysis of several of the primary and trace elements in whole blood. A tungsten filament spiral electrode was used with the CMP, and whole blood samples were deposited on the electrode and subsequently dried, ashed, and atomized. The emission was measured with a spectrometer and either a photo diode array or a charge coupled device detector. A sample size of only 2 μL was required and the time for each sample run was under 4 minutes. This method has a wide dynamic range, allowing the determination of both the primary elements in blood and the elements present in trace quantities.

Much of the initial work focused on measuring the levels of lead in blood. A detection limit of 30 ppb for lead in whole blood was obtained and good accuracy was obtained in the analysis of whole blood standards from the National Institute of Standards and Technology.

The research then focused on applying the CMP-AES to other elements in blood. The elements studied were potassium, sodium, lithium, magnesium, manganese, and zinc. Good linearity was obtained for these elements and the concentration levels obtained for these elements were consistent with literature values.

The primary advantages of this method are that no sample pretreatment or dilution is required, it is easy to run, has a low instrument cost, and is capable of doing multielement analysis.

CHAPTER 1
INTRODUCTION

The research project involved developing a capacitively coupled microwave plasma atomic emission spectrometer (CMP-AES) as a clinical method for multi-element analysis in whole blood. Microwave supported plasmas are an excellent source for atomic emission spectrometry. They produce a high degree of excitation of atomic and polyatomic species, have a relatively low cost, and are simple to operate. CMP's have shown the ability to accomplish direct elemental analysis in complex matrices [1-3]. Direct analysis is desirable because it eliminates the use of hazardous chemicals and the dilution of the sample in trying to minimize matrix effects [1]. Direct analysis also eliminates any contamination of the sample or interference with the plasma that could be introduced by the solvent.

A sample introduction method has been developed that enables the CMP-AES system to directly determine the concentration of several elements in whole blood without any sample dilution or pretreatment. A tungsten electrode is

used which has a spiral loop at the top. The samples of blood are placed on this loop and dried by inductively heating the electrode using microwave power. A flow of helium gas is introduced through a quartz torch that supports the electrode, and a low power plasma is formed at the top of the electrode to ash the blood sample. The power of the plasma is then increased for atomization and excitation of the sample. The resulting emission is measured using a spectrometer and either a photodiode array or a charge coupled device detector. The peak area of the atomic emission line of the analyte is then compared to an analytical curve of standards to determine the concentration of the analyte in the blood.

Initially this research focused on developing a screening method for lead in whole blood. The design of the filament was optimized, as well as the conditions for drying, ashing, and atomizing the sample. All optimizations were done using lead as the analyte. The method worked well with whole blood, and gave excellent linearity and good precision. The accuracy was tested by analyzing blood lead Standard Reference Materials (SRM's). Good agreement was obtained with SRM's with concentrations greater than 100 ppb.

The CMP-AES method was then used for the analysis of several of the medically significant primary and trace

elements in blood. The following elements were chosen for analysis: sodium, potassium, magnesium, manganese, lithium, and zinc. For each element an atomic emission line was chosen that was free from interference and that produced a linear analytical curve over the concentration range of interest. The operating conditions had to be modified to some extent for several of the elements. Analysis was performed on human blood standards for each element sequentially by the method of standard additions. The CMP-AES gave good linearity and precision for these elements. The determined blood levels for most of the elements studied were consistent with those found in literature.

In this dissertation, the development of the CMP-AES for use as a method for elemental analysis in whole blood will be discussed. A brief overview of atomic emission and the microwave plasma as an analytical method will be given. The clinical importance and the methods currently used to analyze the elements studied will also be presented. The main portion of the dissertation will discuss the experimental setup used, the development of the sample introduction system, and the results of the analysis of the selected elements in whole blood. The last chapter of the dissertation will discuss the conclusions made from the research and the possibilities for future work.

CHAPTER 2
BACKGROUND

Atomic Emission Spectrometry

Atomic emission spectrometry is a useful method for elemental analysis. It is very specific, has a wide dynamic range, and has the capability of measuring many elements simultaneously. Typically, its disadvantages include poor sensitivities and serious matrix effects [4].

Atomic emission is the process of an atom being brought to an excited state. The relaxation of the atom from the excited state results in the emission of radiation. The outer shell (valence) electrons are the components of the atom that are excited. The electrons can be excited to a number of different levels. The photons emitted from the electrons as they relax from the different energy levels have characteristic frequencies (ν) giving rise to many wavelengths for each element. The energy levels of each element are different which results in a distinct emission spectrum for each element. The energy (E) associated with each emitted photon is determined by the product of Planck's constant ($h = 6.63 \times 10^{-34}$ Js) and the frequency,

$$E = h\nu = hc/\lambda$$

where c = the speed of light (3.00 x 10^8 m/s in a vacuum). Figure 2.1 gives a simple example of the energies associated with various transitions. The dotted lines represent the excitation of electrons to two different energy levels, and the solid lines indicate the various modes of relaxation with their corresponding energies.

Not all transitions have the same probability of occurrence. In general, the strongest emission is observed from transitions which terminate at the ground electronic level. This is called a resonance transition. If the condition of thermal equilibrium is maintained, then the number density (atoms per cm^3) of analyte atoms in a given excited state (n_i) can be related to the total number density of analyte atoms (n_t) by the Boltzmann distribution:

$$n_i = \frac{n_t g_i e^{-E_i/kT}}{Z(T)}$$

The temperature (T) is the absolute temperature (K). E_i is the excitation energy (J) relative to the ground state, and g_i is the statistical weight of state i. Z represents the electronic partition function:

$$Z(T) = \sum_{i=0}^{\infty} g_i e^{-E_i/kT}$$

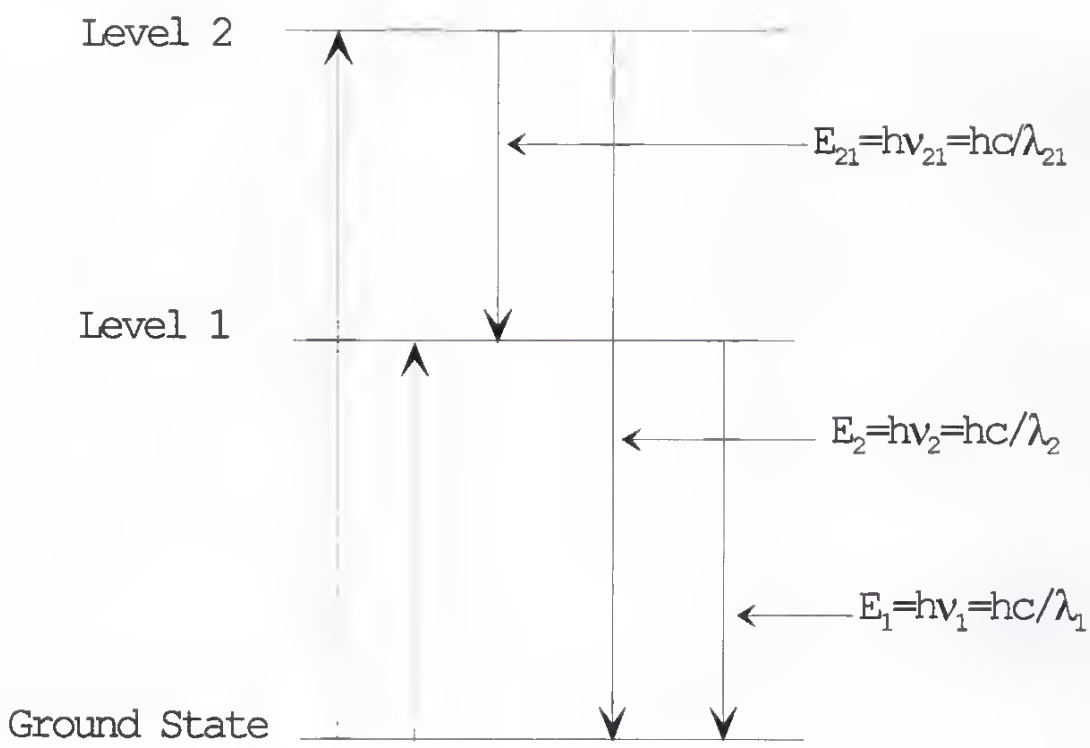


Figure 2-1. Energy diagram for excitation and emission [5].

The radiant power of emission (Φ_E) between two states (from state i to state j) is given by the product of the population density of the excited atoms (n_j), the transition probability (A_{ji}, s^{-1}) that an excited atom will undergo the transition from j to i, the energy of the emitted photon ($h\nu_{ji}$), and the volume element observed (V in cm^3):

$$\Phi_E = n_j h\nu_{ji} A_{ji} V$$

This value, as well as the number density of excited atoms, is proportional to the analyte concentration in the sample. This relationship is good only for low concentrations. By measuring the intensity of emission from standards of various concentrations of the analyte, the exact relationship between analyte concentration and Φ_E can be determined.

Excitation Sources

Many types of excitation sources are used for atomic emission. Generally the excitation source is also the atomization source. In atomic emission, the sample must first be atomized. This is the process of forming free atoms. When the free atoms are formed, they can then be collisionally excited to produce the atomic emission lines.

For many years, flames were the most commonly used atomic emission source because they are simple, reproducible, and inexpensive. The flames used in atomic

emission are formed by the combustion of an oxidant gas and a fuel gas. There are several disadvantages to using a flame as an atomic emission source. The energy of the flame is difficult to control, and it does not generate enough energy to atomize all the elements or to populate high excited states of some transitions.

The introduction of plasma sources for atomic emission spectrometry has significantly improved the detection limits, accuracy and precision for atomic emission spectroscopy. A plasma is a partially ionized gas sustained through an electrical discharge or through a microwave or radiofrequency field [5-6]. Plasmas are advantageous because they have a higher temperature and a less reactive environment than flames. Plasmas also produce a higher degree of excitation generating more atomic emission lines for use in analysis. Inductively coupled plasmas (ICP) are currently the most widely used. Microwave plasmas are also effective atomic emission sources. Two types of microwave plasmas are the microwave induced plasma (MIP) and the capacitively coupled microwave plasma (CMP).

Choice of Emission Lines

The atomic emission lines are spectrally separated by using an optical dispersion device, typically a grating or a prism. The emission lines must be chosen carefully for

optimum signal-to-noise (S/N). The most intense line for the element of interest is not always the best line for analysis. The spectral line chosen must be free from spectral interferences. Interferences may come from the emission of the inert gas used, impurities in the gas, other concomitants in the sample, or the plasma support materials. For analysis in complex matrices, concomitants in the sample can be a significant problem. The resolution of the spectrometer plays an important role in determining how well the spectral line can be distinguished from nearby spectral lines.

Another factor in choosing an emission line is self-absorption. As discussed previously, the emission intensity is proportional to the number density of the excited atoms. At high concentrations, the number density of atoms in the various energy levels can be very high. The atoms present in the lower energy levels can absorb the energy emitted from the relaxing excited states. At high number densities in the lower energy levels, a significant fraction of the emitted energy can be absorbed instead of being detected as emission signal. This is a significant problem if a resonance line is used because the majority of the atoms are present in the ground state. When self absorption begins to occur, the slope of a log-log plot of emission intensity *vs*

concentration will deviate from the desired value of one and approach a limiting value of one half [5]. In conventional flames, the linear concentration range is often no more than two orders of magnitude because of self absorption [7]. When analyzing samples of such a high concentration that self absorption occurs, there are two basic strategies. The first is to dilute the sample to a concentration where self absorption does not occur. The second is to choose a weaker spectral line that gives a linear response over the concentration range of interest.

Microwave Plasmas in Atomic Emission Spectrometry

Microwaves are radio waves in the frequency range of 1.0 GHz and upward [8]. Microwaves have been very useful for applications in radar and communications because of their high frequency and short wavelength. The high frequency of microwaves provides wide bandwidth capability. The wavelength is long enough to penetrate materials, but short enough to allow microwave energy to be concentrated in a small area. This feature has been taken advantage of in microwave ovens [9].

Two methods for transmitting microwave energy from one point to another are the coaxial cable, and the waveguide. The coaxial cable consists of two cylindrical conductors

separated by a continuous solid dielectric. The microwaves travel through the dielectric [10]. Coaxial cables are capable of a large bandwidth and are small in size, but have the disadvantages of high attenuation and cannot handle high powers. The waveguide can be either a circular or rectangular hollow pipe. It is capable of handling high powers with low loss, but is large in size and only has a narrow bandwidth.

The first microwave discharge was observed in the 1940's by electrical engineers and physicists working on radar equipment [11]. It was viewed as a nuisance instead of a potential technological advancement. In 1951, Cobine and Wilbur described some of the features of a microwave plasma [12]. They described the plasma using helium, argon, air, oxygen, and nitrogen as the support gases.
In 1958, Broida and Chapman used a microwave-induced plasma (MIP) to analyze nitrogen isotopes [13]. Kessler and Gebhardt used a capacitively coupled microwave plasma (CMP) to analyze limestone in 1967 [14]. Mavrodineanu and Hughes used a microwave plasma torch in 1967 to view the emission spectra of several elements by introducing solutions into the crater of a graphite discharge tip [15]. Fallgatter *et al.*, examined an argon microwave plasma as an excitation source for atomic emission spectrometry in 1971 [16]. The

development of microwave plasmas has grown over the years because of their high excitation efficiency for both metallic and non-metallic elements, their low background emission, and their low cost [17]. In recent years, microwave plasmas have been applied to many different analytical applications including analysis of solids, biological fluids, and oil [1-3]. Several authors have written extensive reviews of the use of microwave plasmas in spectrochemical analysis [17-22].

Capacitively Coupled Microwave Plasma (CMP)

For a CMP, a magnetron (microwave power tube) generates the microwaves which are conducted through a coaxial wave guide. Within the waveguide, a standing wave is produced which builds up microwave energy that is transferred to the tip of a central single electrode. By oscillating in the microwave field, the electrons gain enough kinetic energy to collisionally ionize the support gas. This produces a flame-like plasma at the tip of the electrode. The plasma that is produced is capable of atomizing and exciting the analyte in a sample. The signal is measured by focusing the emission on the entrance slit of a spectrometer. The multielement emission is usually measured with a photodiode array (PDA) or a charge coupled device (CCD).

Several parameters must be optimized in order to obtain satisfactory results with a CMP. These parameters include the microwave power, the plasma gas flow rate, and the position of the electrode with respect to the detector. Optimum microwave powers differ depending on the type of samples and the method of sample introduction. Helium is used most often as the support gas with flow rates ranging from 3 to 10 L/min [23].

Spencer *et al.* studied various parameters for a high flow rate (>6 L/min) CMP [24]. The temperature measurements were made with the following plasma conditions: 10 L/min helium, 150 cm^3/min hydrogen and 700 W of applied power. The following results were obtained for the analysis of aqueous solutions: excitation temperature = 3430 K; and electron number density = 4.4×10^{14} cm^{-3}. They determined that the values of T_{exc} and n_e are not statistically different for the introduction of aqueous and organic solutions into the plasma.

Microwave Induced Plasma (MIP)

Microwave induced plasmas (MIP) are created by using an external resonant cavity or some other structure to couple microwave energy to a stream of gas in a quartz tube. MIP's are sustained at low powers (25 to 200 W) with argon or

helium as the support gas [18]. A microwave power supply is attached to an antenna or circuit loop by a coaxial cable. The energy goes through the antenna or loop and is introduced into the resonant cavity generating a standing wave. A quartz tube is placed in the cavity in such a way that its axis is parallel to the line of electric field oscillation. MIP's that use an electrothermal type of atomizer have resulted in the best detection limits [18].

Microwave induced plasmas are more widely used than capacitively coupled microwave plasmas because MIP's require lower power and can be operated at atmospheric pressure. In addition, CMP's involve the use of an electrode which can cause spectroscopic contamination and memory effects if it erodes [23]. However, CMP's do have several advantages over MIP's. MIP's can only be operated at low powers while CMP's are stable over a wide range of power levels (50-2000 W). At higher powers, there are fewer matrix effects and more intense signals. Also, a wide range of gases can be used to sustain CMP's and CMP's are more tolerant to the introduction of foreign materials than MIP's [25]. Sample introduction problems have hindered the development of commercial MIP instruments [5]. Memory effects are also a problem in MIP atomic emission spectroscopy [18]. The memory effects are probably a result of etching of the quartz tube by the plasma providing a region where analyte

atoms can collect. An MIP is most useful as an excitation source when it is combined with a separate sample atomizer.

Microwave Plasma Torch

Jin *et al.* developed a new type of microwave plasma called the microwave plasma torch (MPT) [26-32]. A MPT contains three concentric tubes, with the outer tube made of brass and the inner tubes made of copper. The outer tube serves as the microwave cavity which couples the microwave energy to the torch forming a plasma at the top of the torch. The carrier gas containing the sample aerosol enters the inner tube and the plasma gas (helium or argon) flows through the middle tube. This microwave plasma is very stable and has a high tolerance to the introduction of foreign materials [30]. The linear dynamic range for the MPT was generally more than three orders of magnitude and the detection limits for 15 rare earth elements were in the part-per-billion (ppb) range [32]. This is a significant advancement over the MIP because the MPT can withstand the introduction of wet aerosols. Solutions are nebulized by an ultrasonic nebulizer and the resulting aerosol is introduced through a desolvation-dessicator system. The MPT does however suffer from matrix effects and air entrainment in the torch.

Comparison to the Inductively Coupled Plasma (ICP)

The inductively coupled plasma (ICP) is widely used in industry and research. The ICP has a somewhat higher temperature than the microwave plasma and produces a high degree of excitation. The ICP consists of several components. A gas, typically argon, flows through a torch made out of three concentric quartz tubes. The top of the torch is immersed in a high energy induction coil which carries radiofrequency power (at 27 or 40 MHz) in the range of one to three kilowatts. This causes the oscillation of the argon atoms, and the high energy collisions that result produce a plasma at the top of the torch with a temperature more than 6000 K [6,33]. The sample is generally introduced by nebulization [34]. A fine mist of sample is generated by pumping the sample through a pneumatic nebulizer and spray chamber.

Conclusion

Atomic emission spectrometry is a very selective analytical method that can be used for many types of samples. Plasma sources have further increased the usefulness of atomic emission spectrometry. Table 2-1 shows a comparison of the plasma sources discussed. Although the ICP and the MIP are currently in wider use than the CMP, the

CMP is better able to analyze complex matrices. This feature of the CMP can be used for direct elemental analysis in blood.

Table 2-1. Comparison of inductively coupled plasma (ICP), capacitively coupled microwave plasma (CMP), microwave induced plasma (ICP) and microwave plasma torch (MPT) for atomic emission spectrometry [21].

	ICP	CMP	MIP	MPT
Gas	Argon	Helium	Argon or Helium	Argon or Helium
Power (W)	500-1500	70-1000	10-150	40-500
Gas temperature (K)	2000-6000	2000-3500	500-2000	1000-6000
Relative standard deviation	0.5-2%	2-10%	0.5-2%	1-5%
Linear dynamic range	~10^5	~10^4	~10^3	~10^4
Limit of detection (ppb)	0.1-100	0.1-1000	0.1-100	0.1-100

CHAPTER 3
CLINICAL ELEMENTAL ANALYSIS IN BLOOD

Introduction

The human body requires a delicate balance of the levels of various elements. Too much or too little of a particular element can have devastating physiological effects. Some typical ailments are a result of an imbalance of elements in the body. High levels of sodium and low levels of potassium, magnesium, and calcium all lead to hypertension (high blood pressure) [35]. Hypertension is the most common disease in industrialized societies and contributes to the development of cardiovascular disease, stroke, and renal failure [35]. The reduction of the levels of certain elements caused by medical treatment with some drugs can also cause serious problems [36].

Elements are transported by the blood and taken up in varying amounts by organs and tissues [4]. The significance of the levels of various elements in health makes it important to have readily available techniques to monitor these elements. The biological importance of each element

studied during the course of this research and the methods used to measure them will be discussed.

Medical Significance

Trace Elements

An element is classified as a trace element if the concentration is below 250 ppm [37]. Trace elements can be classified into two groups, essential and nonessential. An element is considered essential if lack of that element causes problems. Essential trace elements include manganese, copper, zinc, tin, and nickel. Nonessential elements are those that are present in biological organisms but have not been determined to play an important role [4].

The role of trace elements in the body has received much attention by the scientific community. Although they are present in low concentrations, they can play essential roles in biological functions, and can also be detrimental to biological activity if present in too great an amount [14]. Trace elements are very important in the structure of enzymes and are needed in the production of proteins. Trace elements are also essential for the normal growth and development of the human skeleton [36].

The metabolism of certain trace elements is involved in various diseases. Therefore, the measurement of the

concentration of trace elements in biological fluids can be used as a test for certain diseases. Poor health can also be caused by environmental exposure to some elements. The proper levels of trace elements are especially important during pregnancy to insure a healthy child [36], and in the elderly for their immune response [38]. Trace element losses must be monitored closely in patients receiving radiation therapy or chemotherapy. These patients may require additional supplements of certain elements to compensate for losses due to significant weight loss from their illness or treatment [39].

Lead

Lead (Pb) poisoning is the leading environmental threat to children in the United States [40-41]. The primary sources of lead exposure are lead based paints and lead-contaminated dust and soils. The Department of Housing and Urban Development estimates that 4 million homes containing young children have lead-based paint hazards [42]. Children can also be exposed to lead though air, water, and food.

Lead poisoning affects virtually every system in the body. It is especially harmful to the developing brain and nervous system of unborn and young children [40, 43]. Lead causes health problems because the body is unable to distinguish between lead and calcium. When a person consumes lead it is assimilated into the blood stream the

same way calcium is. Young children and pregnant women absorb calcium more efficiently to meet their added requirement so they are particularly at risk. Typically, adults absorb 10 to 15 percent of the lead that reaches their digestive tract. Pregnant women and young children can absorb as much as 50% of the lead [44].

In blood, 94% of the lead is bound to the hemoglobin. Within the past five years, it has been found that lead concentrations as low as 100 ppb in the blood can be detrimental to the health and intellectual development of a child [40, 45]. Figure 3-1 shows the health effects of various levels in the blood [40]. Acute lead poisoning can result in anorexia, dyspepsia and constipation followed by abdominal pain [46]. The detrimental effect of lead poisoning on young children has led the Centers for Disease Control and Prevention (CDC) to lower the acceptable level of lead concentrations in the blood of children to 100 ppb, compared to a level of 250 ppb considered acceptable from 1985 to 1991. The symptoms of lead poisoning are often invisible at first, preventing the diagnosis and treatment of most cases. The number of lead poisoning cases can be greatly reduced if a large scale screening program is implemented. This would require an inexpensive, easy-to-use method to detect trace amounts of lead in blood.

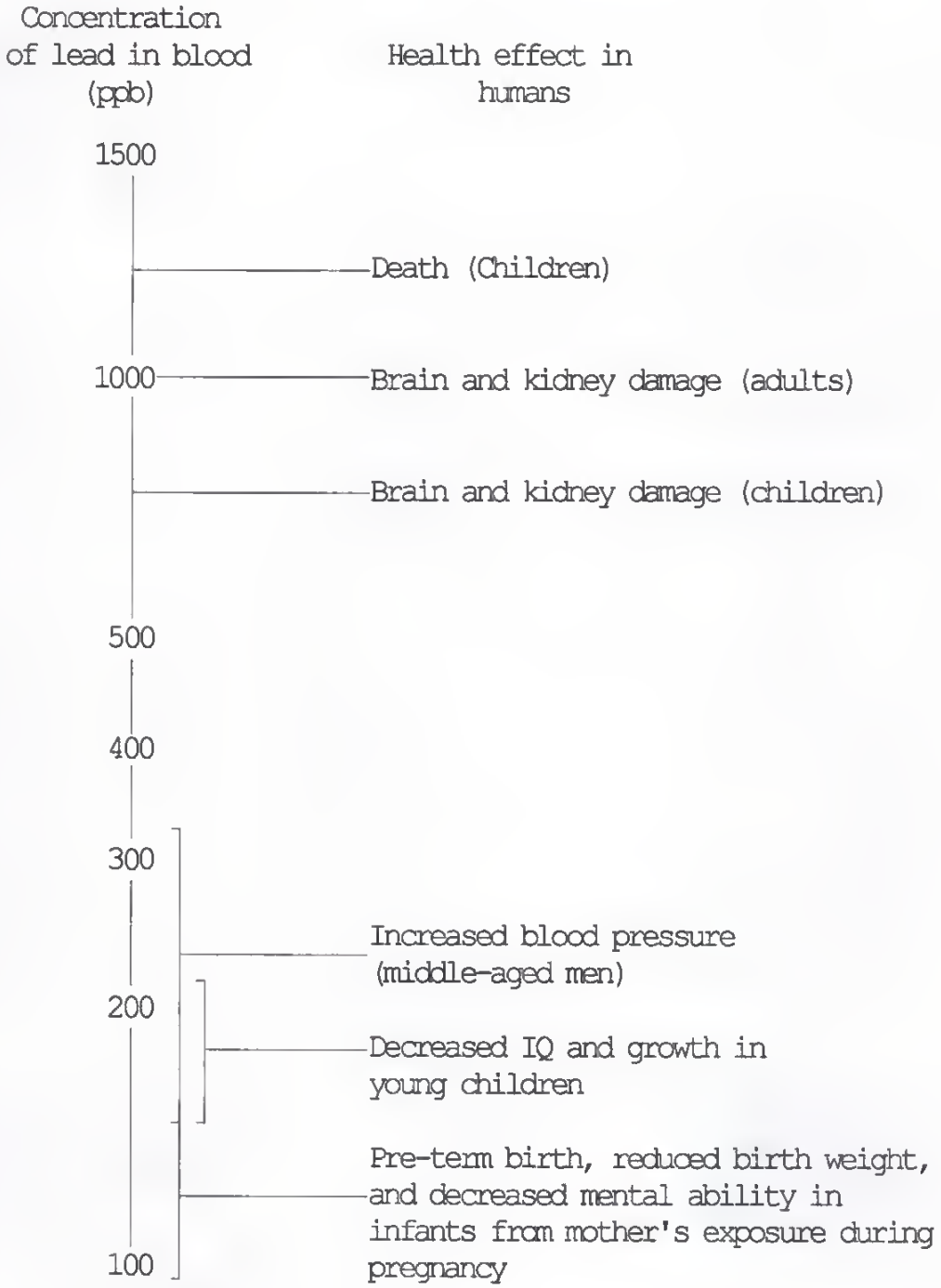


Figure 3-1. Health effects of lead poisoning [40].

Manganese

Manganese (Mn) is a important as a constituent of metalloenzymes and as an enzyme activator [37, 47]. Research with animals has shown that Mn deficiency can lead to impaired growth, skeletal abnormalities, disturbed reproductive function, and problems with lipid and carbohydrate metabolism. A deficiency of manganese leads to a decreased level of blood clotting proteins and has also been observed in several diseases including epilepsy [47-48].

Toxic levels of manganese can be the result of chronic inhalation of airborne particulates containing high concentrations of Mn from mines, steel mills, or some chemical industries. Patients with liver disease are also at risk from Mn toxicity because their liver may not adequately clear the Mn absorbed from a normal diet. The main signs of Mn toxicity include depressed growth and appetite, impaired iron metabolism, and altered brain function. Severe psychiatric abnormalities including hyperirritability, violent acts, and hallucinations can be caused by Mn toxicity [37].

Lithium

Lithium (Li) is used in the treatment of manic depressive psychosis [49]. Lithium is administered in the form of lithium carbonate or another lithium salt with as

much as 1800 mg taken daily [50]. Blood normally only contains lithium at a level of low ppb, but for therapeutic lithium levels, a range of 0.5-1.5 mM is maintained in the blood [51]. This is close to the toxic level, and a level of 5 mM can be lethal. This necessitates the monitoring of lithium levels in patients receiving this type of treatment.

Zinc

Zinc (Zn) is the second most plentiful trace element in the body. Zinc is important in the metabolic functions of the body and is essential for the production and functioning of over 40 enzymes that contain zinc [36]. Zinc is also vital in the synthesis of DNA and RNA in every living cell. Zinc plays a role in immune functions, in growth and development, and in the synthesis and release of testosterone. Zinc is especially important in expectant mothers and in the growth of young children [52-53].

Zinc plays a major role in fighting infections and in the healing process [47]. Those at risk for zinc deficiency include women of child bearing age, young children, and the elderly [54]. If the zinc level is too low it can cause congenital malformations, including spina bifida and central nervous system abnormalities. It can also cause severe growth retardation, arrested sexual maturity and a loss of appetite [36]. The level of zinc is especially critical in the elderly because of the deterioration of immune function

with age. Low zinc levels can indicate diabetes because zinc is important in the storage and release of insulin. A zinc imbalance may be involved in hypertension.

Most cases of zinc toxicity have been related to food poisoning incidents and to industrial pollution [53]. Too much zinc causes anemia (reduced hemoglobin production), elevated white blood cell count, muscular problems, exhaustion, diarrhea, nausea and dizziness [52-53]. Very high levels of zinc can impair metabolic functions that are dependent on other trace elements [54]. High levels of zinc can also interfere with the absorption of copper which can provoke iron deficiency and anemia [47].

Magnesium

Magnesium (Mg) is essential in the transfer, storage, and utilization of energy. Mg regulates and catalyzes over 300 enzyme systems in mammals [55-56]. Mg also maintains the cardiovascular system, regulates DNA and RNA synthesis and structure, and is important in cell growth, reproduction, and membrane structure. Mg controls many processes in the body including neuronal activity, neuromuscular transmission, cardiac excitability, muscular contraction, blood pressure, and peripheral blood flow [47, 55].

A deficiency of Mg promotes hyper coagulability of blood, atherogenesis, vasoconstriction, cardiac arrhythmias

and also damage to the cardiac muscles. A Mg deficiency may also be related to cardiovascular disease, hypertension, diabetes, depression, and atherosclerosis [35, 52].

Major Elements

Sodium

Sodium is the principal cation of the extracellular fluid. It is essential in maintaining the pH balance of the fluid and is also important in nerve transmissions and muscle contraction. Sodium levels can be depleted by vomiting, diarrhea, or heavy sweating. If depletion occurs it is critical to take measures to bring the sodium level back up to a healthy level. If the sodium level is too high it can result in hypertension, kidney disease, or heart disease [47].

Potassium

Potassium is important in the body in its maintenance of fluid and electrolyte balance and cell integrity. Diabetic acidosis, dehydration, or prolonged vomiting or diarrhea can cause low potassium levels. Symptoms of low potassium levels are muscular weakness, paralysis, and mental confusion. Too much potassium can also cause muscular weakness, confusion, as well as numbness, slowed heart rate, vomiting, and eventually cardiac arrest [47].

Methods of Analysis

Lead

The Centers for Disease Control and Prevention has set forth a number of desirable characteristics for an improved blood lead measurement system. These characteristics include an accuracy and precision of ±10 ppb at 100 ppb, a detection limit of 10-20 ppb, a sample volume of less than 200 μL, a low cost-per-test, an analysis time under five minutes, portability, and minimal operator training required to perform the method.

Screening methods

Currently used screening methods for lead in blood which measure the level of either erythrocyte protoporphyrin (EP) [57] or zinc protoporphyrin [45] in blood as an indication of lead poisoning are not sensitive enough to measure blood lead levels below 250 ppb. The EP test is based on the increase in the amount of EP caused by an increase in Pb. Porphyrins are the metabolic intermediates in the biosynthetic process that produces heme [52]. Lead impairs heme synthesis, preventing the incorporation of iron into the protoporphyrin. This allows free protoporphyrin to chelate cytosolic zinc. The amount of free protoporphyrin can be measured because it fluoresces deep red [58]. The whole blood is diluted and matrix modifiers are added. The

porphyrins are then separated from the blood and measured by molecular fluorometry. This test has been recommended by the CDC since 1978 [57]. Hematofluorometers have also been used to screen children for lead [59]. These are portable instruments that measure the zinc protoporphyrin directly in a single drop of blood.

Two current methods being developed as portable screening methods are anodic stripping voltammetry (ASV) [60-65] and potentiometric stripping analysis (PSA)[66-67]. In anodic stripping voltammetry, a decomplexing agent is added to the blood sample to free up the lead for electrolysis. The Pb is reduced at a controlled potential causing it to plate out on the surface of a mercury electrode. A voltage sweep of the electrode releases the lead and produces a current between the working and reference electrode. By measuring this current, the amount of lead can be determined [64]. This method has the problems of instrument instability, slow speed, and variations in response due to other elements present in the blood. ASV also requires a plating solution. The use of ASV with microelectrode arrays and indium as an internal standard has improved the detection limit and precision for the analysis of lead in blood [61].

In potentiometric stripping analysis, the lead analyte is preconcentrated in a mercury film on a glassy carbon

electrode. This occurs by potentiostatic deposition where electrons are added to the metal. The stripping step is then achieved chemically by adding an oxidant. During the stripping, the potential of the working electrode as a function of time is closely monitored. This will produce a well-defined stripping plateau which can be used for the analysis of lead. Whole blood has to be diluted by a factor of ten for analysis by the method of standard additions [67]. The total time for analysis is about 5 minutes.

Both ASV and PSA possess the required accuracy and precision to detect low blood lead levels. Electro-chemical methods are advantageous as a screening method because they are both portable and inexpensive; however, they have the disadvantage that they require the use of reagents and sample pretreatment when analyzing whole blood.

Exeter Analytical (North Chelmsford, MA) has developed a commercial atomic absorption spectrometry (AAS) instrument that can be used for blood lead screening [68]. The lead absorption line at 283.31 nm is used with near line background correction using the non-absorbing 287.33 nm lead line. This instrument used a 150 W tungsten coil filament in an enclosed chamber. Tungsten coils are excellent atomization sources because of their high heating rate and their commercial availability. Tungsten coils that are made for halogen projector lamps can be used so they are

relatively inexpensive. One coil can last for approximately 70 runs. The blood samples were diluted by a factor of ten with 0.2 % nitric acid, 0.5% Triton x-100, and 0.2% $NH_4H_2PO_4$. Calibration is done with aqueous standards, and a detection limit of 30 ppb with a RSD of 9.0% at 100 ppb is obtained. This method produces results in less than 3 minutes, has a low cost-per-test, and is easy to operate [68].

Recently, a portable, battery powered AAS was developed by Jones and coworkers for lead in blood screening [69]. A tungsten coil was used as the atomizer and a miniature fiber optic spectrometer with a charge coupled device (CCD) mounted on a input card of a personal computer was used as the detector. The blood samples were digested in nitric acid by microwave heating and then diluted with distilled deionized water. A 20 μL sample was placed on the coil and then dried for 2 minutes at 3.0 A and then atomized at a current of 6.0 A. The absorption signal was collected using a hollow cathode lamp and a fiber optic. The spectrometer and multichannel detector allowed near-line background correction technique to be used. The lead absorption line at 283.3 nm was used for analysis and the average of the nonabsorbing lead lines at 280.2 and 287.3 nm was used for background correction. The total cost of this entire system was below $6000. A detection limit of 1 ppb for lead was determined. The linear dynamic range was 2 orders of

magnitude and the precision was 5%. The method was proven to be accurate by analyzing NIST blood standards. The coil was used in the analysis of up to 400 samples [69].

Clinical methods

Research is being done by many different government agencies and universities to improve blood lead measurement. Isotope dilution inductively coupled mass spectrometry (ID-ICP-MS) [70] and graphite furnace atomic absorption spectrometry (GFAAS) [71-72] are methods that are able to detect trace amounts of lead in blood below the level of concern (100 ppb). Both of these methods are very accurate and precise, but have the disadvantages of requiring sample pretreatment and expensive instrumentation. The expense of testing is a major consideration since millions of children would need to be tested in a large-scale public health screening program.

Atomic absorption spectrometry (AAS). Graphite furnace atomic absorption spectrometry (GFAAS) is one of the most popular methods for lead in blood analysis [71-90]. GFAAS has excellent sensitivity and selectivity, large throughput, and is capable of analyzing very small volumes. Many GFAAS methods use a L'vov platform which is a small platform placed in the graphite tube to hold the sample and ensure that the tube and sample come to the same temperature at the same time. In 1991, the Centers for Disease Control (CDC)

surveyed the methods being used by clinical laboratories for blood lead analysis. Of the laboratories surveyed, 61% used GFAAS, 5% of the labs used Delves cup AAS, 7% used extraction AAS, 1% used carbon rod AAS, and 26% used ASV [81].

The methods used to analyze lead in blood by GFAAS include: direct introduction of blood into the furnace; dilution with either water, Triton X-100, or acid; deproteinization with nitric acid; matrix modification; solvent extraction; or a combination of several methods [82]. The direct injection of blood samples into a graphite furnace has many problems associated with it. The blood can seep into the graphite and produce major memory effects [83-84], and during drying and atomization, the blood residue can cloud the viewing windows [85]. Also, a carbonaceous residue from the proteins in the blood builds up in the furnace and is unable to be vaporized even at high temperatures [76, 83]. Diluting the blood samples with water alone is not sufficient to reduce adequately the amount of carbonaceous residue [86]. The presence of water in the blood sample also gives rise to a slow precipitation of the red cell membranes, reducing the homogeneity of the sample [87]. Diluting the blood samples with a 0.5 to 2% solution of Triton X-100, a surfactant, causes complete

lysis of the blood cells and produces a clear solution that minimizes the negative effects of the blood matrix [88].

The problem of carbonaceous residue build up can be virtually eliminated by deproteinization of the blood with 30-50% nitric acid. The supernatant of the resulting sample can then be injected into the graphite tube. This procedure destroys the bulk of the organic matter in the blood. However, the use of nitric acid shortens the life of the graphite tube because of the oxidation of the tube's pyrolytic coating [89]. The blood could be deproteinized at lower concentrations of acid, but the inorganic salts present were removed, necessitating the use of standard additions [90].

Adding matrix modifiers to the blood can help in retaining the analyte while volatilizing away most of the matrix. The most common matrix modifiers used in blood lead analysis are diammonium hydrogen phosphate, ammonium dihydrogenphosphate, and phosphoric acid [75, 82]. By adding these matrix modifiers, higher furnace temperatures can be used to ash away the matrix without significant loss of the analyte. The method of solvent extraction can also minimize matrix effects, but it is very tedious, prone to contamination, and does not completely remove interferences [91-92].

A GFAAS method has been developed which allows aqueous standards to be used for blood lead analysis [71]. Prior to analysis, the blood is deproteinized with a 5% nitric acid solution containing 0.1% Triton X-100. The supernatant is collected and the concentration of lead is measured using Zeeman GFAAS. Parsons and coworkers have also developed a method capable of calibrating with aqueous standards [45, 75]. A transversely heated graphite tube/platform called a stabilized temperature platform furnace (STPF) was used. This method produced a nearly isothermal system which reduced the time of analysis, increased the precision, and eliminated many of the chemical and matrix interferences. Blood samples preserved in EDTA were diluted by a factor of 10 with a solution containing ammonium dihydrogen phosphate, triton X-100 and nitric acid. The samples were directly introduced into an autosampler where the mixing with the solution occurs. Twelve micro-liter aliquots were injected into the furnace and atomized. Each analysis took 90 s, and the system was able to run approximately 100 samples per day with duplicate injection. The precision was better than 5%. While both of these methods are advantageous because aqueous standards can be used for calibration, they have the disadvantage of requiring appreciable sample treatment.

A flame AAS method has been developed that used 20 μL of blood samples spotted on filter paper and then analyzed

in a Delves cup [93]. A Delves cup is a small nickel cup that is positioned in the flame for analysis. The blood sample must be allowed to dry on the filter paper and is then ashed. The ashing step burned away the paper and then the sample was introduced into the flame to be analyzed for lead by measuring the absorption at a wavelength of 283.3 nm. The entire analysis time was 15 s per sample and a limit of quantitation of 40 ppb was obtained. This method gave excellent reproducibility and accuracy [93]. It has the disadvantage that there was considerable variability in the adsorptiveness of the papers which was detrimental to the accuracy. Also, this method's requirement of allowing the blood to dry on the filter paper resulted in the sample being susceptible to contamination from airborne particles. As a clinical method, flame AAS has the disadvantage that the equipment is expensive and cumbersome and requires a combustible gas source.

Inductively coupled plasma atomic emission spectrometry (ICP-AES). A carbon rod atomizer has been used to analyze blood samples with a ICP atomic emission spectrometer [94]. Blood samples were diluted by a factor of five with distilled water. The samples were placed on the carbon rod atomizer and then dried and volatilized. The resulting vapor was carried into the plasma by the plasma gas. This method of sample introduction was more efficient than

nebulization. An aqueous detection limit of 7 ppb was reported for lead with a relative standard deviation (RSD) of 0.2%.

Inductively coupled plasma mass spectrometry (ICP-MS). ICP mass spectrometry is a very sensitive method for the measurement of lead in blood [70, 95-96]. The main method of sample introduction in an ICP-MS is a nebulizer. Aqueous samples are transferred to a nebulizer by a peristaltic pump. The aerosol produced by the nebulizer is carried to the plasma by a flow of gas, typically argon. The high temperature of the plasma vaporizes and ionizes the sample and the ions are then detected in a mass spectrometer according to their mass to charge ratio [97]. ICP-MS with isotope dilution, is the method with lowest bias for determining lead in whole blood and serum [70, 95]. Isotope dilution mass spectrometry involves measuring the change in the relative abundance of two isotopes of an analyte after adding a known amount of one of the isotopes to the sample. The CDC uses isotope dilution (ID) ICP-MS for the analysis of its certified reference material, lead in bovine blood, from its Blood Lead Laboratory Reference System. An aliquot of the whole blood sample is spiked with a radiogenic lead isotopic standard. This aliquot along with an unspiked aliquot is then digested with ultrapure nitric acid in a microwave oven. After cooling, both samples are diluted and

then aspirated into an ICP-MS. The isotope ratios of lead at mass 206 and mass 208 are then measured. While this method is very accurate and precise for determining lead in blood, it is more suitable for determining reference values than being used as a clinical method because of it's high cost and low throughput (10 samples per day) [70, 95].

Primary and Trace Elements

The main methods for trace elemental analysis in the clinical laboratory are absorption or emission spectrophotometry. Typically, the blood is separated, and the plasma or serum is used for analysis [4]. Methods capable of performing trace elemental analysis include AAS, ICP-AES, and ICP-MS. Other methods include electrochemical, neutron activation, flame atomic fluorescence spectrometry, molecular absorption spectrometry, X-ray fluorescence, particle-induced X-ray emission and radiochemical techniques [46]. However, many of these methods are not suitable for routine use in a clinical setting. Neutron activation, for example, is a very sensitive technique but requires the use of a nuclear reactor and requires a very long time for analysis [98]. Currently, AAS is the most widely used method in clinical laboratories, usually employing electrothermal sample introduction [46]. Recent reviews of clinical methods of analysis have appeared in *Analytical*

Chemistry [99] and in the *Journal of Analytical Atomic Spectrometry* [100].

Atomic absorption spectrometry (AAS)

Sodium, potassium, zinc, magnesium, and iron blood levels can be determined by flame atomic absorption spectrophotometry (FAAS) [47, 101]. The samples are diluted and introduced into the flame. The analysis of each element requires a hollow cathode lamp that produces light at a wavelength specific for that element. The fraction of absorbed light is used to determine the concentration of the element present. Shang and Hong have used a microvolume injection technique to measure the levels of Cu, Zn, Ca, Mg, and Fe by FAAS [102]. The blood samples were treated with triton x-100 and then diluted with a mixture of 0.18 M HCl, 0.003 M La_2O_3, and 0.013 M KCl. The injection volume used was 10 μL. Atomic absorption has greater sensitivity than either flame atomic emission spectrometry (FAES) or ion selective electrodes (ISE), but it is less precise and not as suitable for routine clinical analysis. It has a high initial cost and the necessity for compressed gases and flames are undesirable in the clinical laboratory.

GFAAS is a very popular method for elemental analysis in blood. The various methods used for lead analysis are also used for many other elements and have the same

advantages and disadvantages [90]. The levels of magnesium, manganese, lithium and iron have all been determined by GFAAS [47, 50, 52]. GFAAS has achieved a detection limit of 2 ppb for manganese in blood and is the most common method for analysis of lithium in blood [50, 103]. The main disadvantage of GFAAS as a clinical technique is its limitation as a single element technique. Some researchers have developed complex methods of determining two or three elements simultaneously, but it is difficult and expensive, requiring a complicated optical setup [74].

Atomic emission spectrometry (AES)

Sodium and potassium in serum are usually analyzed by either flame atomic emission spectrometry (FAES) or by ion-selective electrode potentiometry (ISE) [101]. FAES requires a dilution of the sample by 100 to 200 times, often adding lithium or cesium to the sample as an internal standard and ionization suppressant. An air-propane flame is used, and the sodium emission is monitored at 589 nm and the potassium emission at 766 nm. Only 1 to 5% of the atoms in the flame are excited to emission, but the concentration of the elements is sufficient for accurate and precise measurements [101]. Lithium levels can also be reliably measured using flame emission spectrometry [104].

Flame photometric flow-injection analysis has been successfully used to simultaneously measure the levels of

lithium, sodium and potassium in blood serum [105]. The serum samples were diluted ten-fold with doubly-distilled deionized water. The sample was then injected and split into three portions so that each portion reached the detector at a different time. Between the analysis of each sample portion, the filter on the detector was changed to be specific for each analyte. This method allowed the analysis of 108 samples per hour [105].

ICP-AES has been used to measure the levels of Fe, K, Mg, Na, Li and Zn in human serum and blood [106-108]. Serum samples were digested in nitric acid or diluted with deionized water. A microsampling system has been developed for ICP-AES which uses <0.1 mL of sample [107]. By digesting the blood or serum sample with acid, aqueous analytical curves could be used for calibration.

Spectrophotometry

Spectrophotometry involves selectively complexing and separating an analyte using either an inorganic or organic colorimetric reagent. Various organic reagents have been used as spectrophotometric agents for the analysis of lithium, magnesium, and iron in blood and serum [51-52]. Calmagite, methylthymol blue and formazam dye, are some examples of chromophores that have been used for the analysis of magnesium. The level of iron in blood is analyzed by exposing the blood sample to strong acids to

dissociate the iron from its binding proteins. A chromogen is then added to the sample to produce a iron chromogen complex that has an absorbance maximum in the visible region. The concentration of lithium in serum can be measured by observing shifts in the spectrum of a reagent caused by the presence of lithium. The reagents must be very specific for lithium because sodium, which is present at high concentrations in blood, is generally an interferent. Crown ethers can be used for lithium analysis. By using different cage sizes, conformational flexibility, and various side groups, crown ethers can be made to form a complex selectively with the several analytes of interest. The complex formed can be extracted into an organic solvent with an anionic reagent that is colored allowing spectrophotometric analysis [51].

A major disadvantage of spectrophotometry is the limited selectivity due to overlapping absorption bands. It is, however, easy to use, rapid, and can be readily automated [4].

Inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS has been used for the measurement of trace elements in whole blood and serum [33, 109-112]. The advantages of using ICP-MS include high throughput (40 samples/hour), possibility of simultaneous analysis, and good detection limits. Over 50 elements have detection

limits in the range of 0.01 to 0.1 ppb [33]. Adding an internal standard can often correct for matrix effects and instrument drift.

Blood and serum samples for ICP-MS are usually digested with acid or diluted. The sample pretreatment often includes a separation step. The amount of time needed for sample preparation has been reported as 25 minutes for 50 specimens [33]. Barany and Bergdahl reported on a method for ICP-MS of trace analysis in blood where whole blood was diluted 50 to 100 times with an alkaline solution. Each analysis required only 75 seconds. Even with dilution, some problems were encountered with the buildup of denatured proteins from the blood so the torch required occasional cleaning. This method was used for the determination of 7 trace elements in blood. It was not suitable however for Mn, Se, Hg, or Cr [113].

The major disadvantage of using ICP-MS is the high cost of the instrument and the operator expertise needed. Also, the analysis of lighter elements is difficult because of more interferences. Interferences arise from mass overlap from either polyatomic ions, doubly charged ions, or elements with the same isotopic masses. Currently, it is not possible to analyze chromium, manganese, or iron by ICP-MS in biological samples due to the presence of interferences [33, 109-110, 113].

X-ray fluorescence

X-ray spectrometry involves bombarding the sample with radiation of distinct energy. This removes electrons from the inner shells forming atoms in an excited state. The electrons from the outer shells fall into the shells vacated by the removed electrons according to specific transition rules. The radiation emitted by this process is very characteristic. The method of x-ray fluorescence can be used for simultaneous multielement analysis on a very small sample of blood (2-3 μL) without destroying the sample [114-115]. The blood levels of potassium, calcium, chromium, iron, nickel, zinc, selenium and lead can all be determined in one measurement. Detection limits ranged from 21 ppm for phosphorus to 30 ppb for lead in blood [114-116]. The method of X-ray fluorescence has the disadvantage that it is very difficult to match the composition of the calibration standards to the matrix of the sample [4].

Electrochemical techniques

Voltammetry, an electrochemical method, is also capable of measuring trace elements in blood. In voltammetry, the measurements are based on the potential-current behavior of a small electrode that is easily polarized [4]. Voltage is applied to a microelectrode and the diffusion current is measured as a function of the voltage. This allows both quantitative and qualitative analysis of the trace element.

For this method, it is necessary to digest completely the samples prior to analysis [4].

Ion selective electrodes (ISE) are capable of determining the level of potassium, sodium, magnesium and lithium in blood or serum by measuring the potentiometric charge as a function of ion concentration [51, 55, 117-120]. The membranes of ISE's are ideally sensitive to only one ion. Most membranes, however, respond to ions other than the one for which they are designed. Polymer-bound liquid membranes use a membrane that contains a sensing material dissolved in the polymer support matrix. If the sensing material is neutral in charge, then it must complex with the analyte in some way to transfer it across the membrane or it must be able to facilitate ion exchange. Neutral sensing materials are called ionophores and are often some type of crown ether. Crown ethers can be made in such a way that they can selectively complex a given ion. The polymer matrix containing the sensing material is often polyvinyl chloride (PVC) [121]. Bulky crown ethers used in a PVC membrane ISE can exhibit a selectivity up to 2000:1 for lithium [51]. A glass ion-exchange membrane is used for the analysis of sodium, and a valinomycin neutral-carrier membrane is used for potassium [101].

The use of ISE's to analyze clinical samples involves either the direct analysis of undiluted samples or the

indirect analysis of pre-diluted samples. Direct ISE methods are subject to bias because of the difference in the serum matrix and the aqueous samples used for calibration. Indirect ISE is susceptible to error introduced by the dilution.

ISE's to monitor Mg can yield rapid results on blood, plasma, serum and aqueous solutions with sample sizes ranging form 100 to 200 μL [55]. The Mg ISE's employ ionophores using neutral carrier based membranes with excellent precision reported at 2 to 4%. However, this method does experience problems with very low levels of magnesium because the analytical response is not linear at these low concentrations [52].

ISE's compare favorably to the methods of atomic absorption spectrometry and flame emission spectrometry for the analysis of several elements. ISE's have the advantages that they function in turbid solutions, have a wide dynamic range, have a rapid response, are inexpensive, and are very portable with current instruments weighing between 7 to 12 kg [117]. The rapid response is very beneficial in monitoring dosages and compliance with medical treatment such as lithium treatment in psychiatric patients. ISE's have the disadvantages that they have limited sensitivity, are subject to interferences from other ions and memory effects, and require frequent calibration.

Conclusion

The analysis of the primary and trace elements in blood is very important in maintaining and monitoring the health of individuals. Although there are many methods capable of doing multi-element analysis in blood, there is still much room for improvement. The most accurate and precise methods all require some sort of sample pretreatment. Sample treatment requires time and is a possible source of contamination. Ideally a clinical method for blood analysis would be able to analyze whole blood directly, without sample pretreatment, and would be able to use simple standards (i.e. aqueous) for calibration.

CHAPTER 4
EXPERIMENTAL SETUP AND MATERIALS

Setup

The experimental setup is shown in figure 4-1. Each component of the experimental setup will be described.

Microwave Plasma Electronics

The microwave plasma was generated by an 870 W magnetron (Samsung OM75A) at 2450 MHz. This type of magnetron is commonly found in domestic microwave ovens. Magnetrons are capable of high power with low cost and high efficiency. Magnetrons produce microwaves through the combination of an anode, cathode, and magnet. Electrons are emitted from the cathode and are introduced into a combination of electric and magnetic fields which cause the electrons to move around the cathode. The electrons then move toward the anode and exchange potential energy, building up the microwave field. When the electrons hit the anode, the power is coupled directly to the output. The output allows the microwaves to be taken out *via* an external

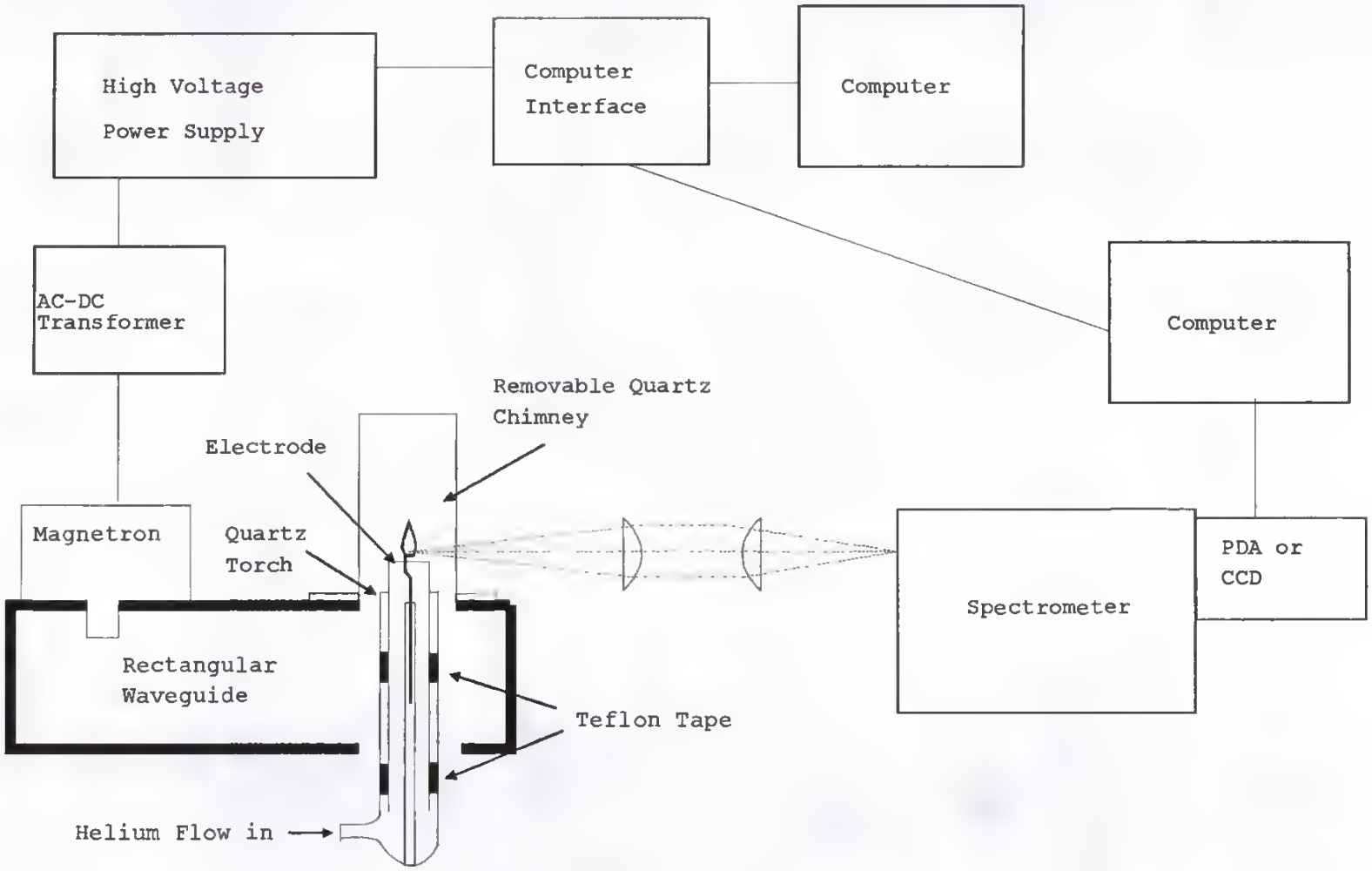


Figure 4-1. CMP-AES experimental setup.

transmission line [9]. A diagram of the magnetron is shown in figure 4-2.

The magnetron was powered by a current regulated analog-programmable power supply (Model 106-05R, Bertran High Voltage, Hicksville, NY, USA). An A.C. power transformer (Magnetek Triad, model F-28U, Newark Electronics, Chicago, IL) was used to provide a high current, low AC voltage for the magnetron filament.

Waveguide

The rectangular waveguide was made out of aluminum and constructed in the laboratory. The waveguide had the following dimensions: height = 47 mm, width = 98 mm, length = 277 mm. The waveguide had a hole near one end on the top allowing the output of the magnetron to be inserted, and holes on the top and bottom near the other end allowing the torch to be suspended within the waveguide. The hole diameter for the torch was 44 mm, and the center of the hole was 58 mm from the end.

Torch

The torch consisted of four concentric quartz tubes: an outer quartz tube (outer diameter (o.d.) = 19 mm) directed the flow of helium; a removable quartz tube (o.d. = 15 mm) reduced the dead volume of the torch; and an inner

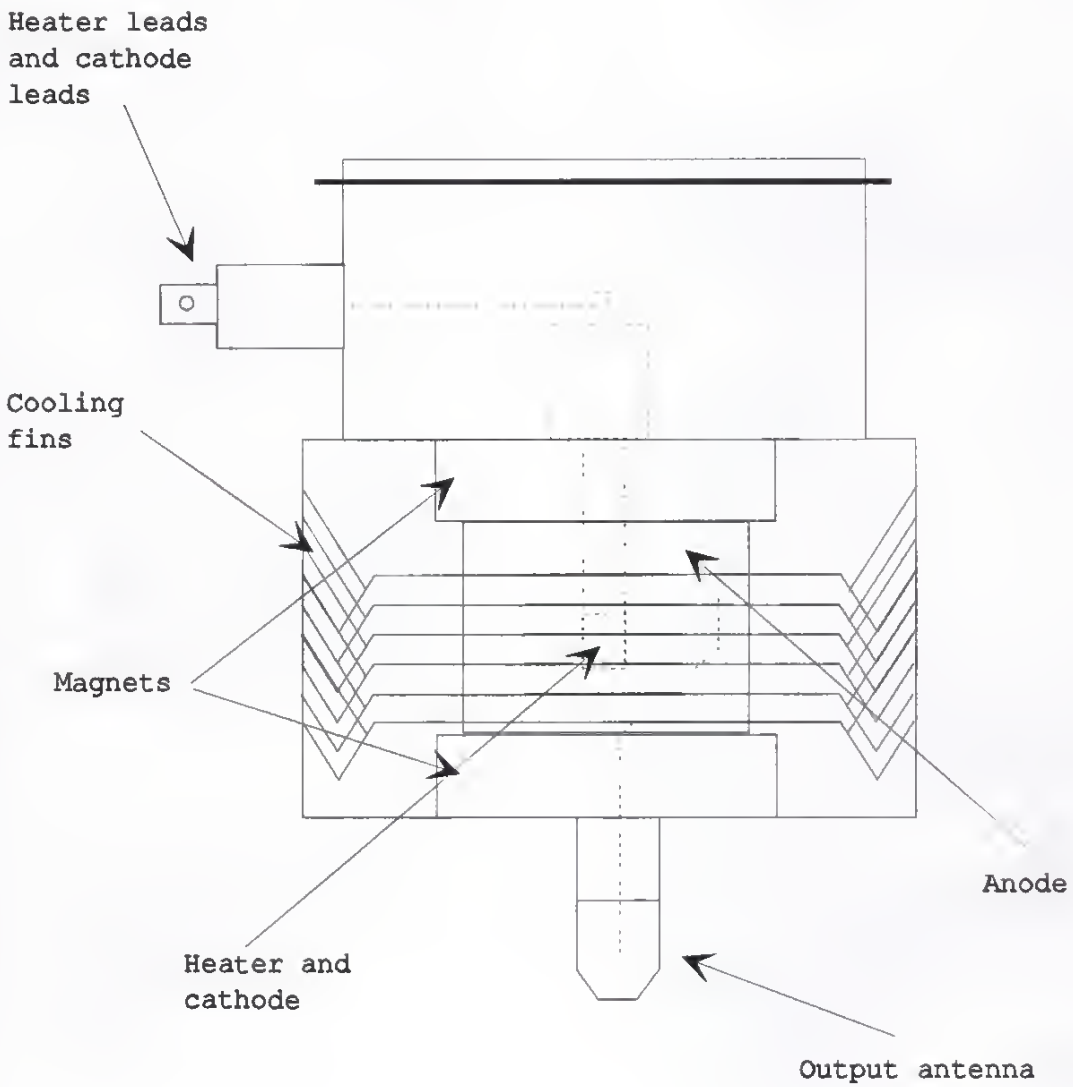


Figure 4-2. Magnetron

quartz tube (o.d. = 5 mm) that supports a short piece of quartz tubing (o.d. = 2 mm) in which the filament rests. The inner quartz tube is used to decrease the volume of the torch in order to reduce the amount of helium gas required. The inner quartz tube is a separate piece of quartz tubing held in place in the torch with teflon tape. In some experiments, the inner tube is brought up around the plasma so that it shields the plasma. The inner tube as a shield is better than using the torch itself because the inner quartz tube is easily replaced if the plasma attacks it and makes it optically unclear. A quartz chimney surrounds the top of the torch to reduce instabilities caused by air currents.

Plasma Gases

Helium (BOC Gases, The BOC Group, Inc., Murray Hill, N.J.) was used as the plasma gas. Helium was an excellent plasma gas for atomic emission spectroscopy because of its high ionization energy [122]. The ionization energy of helium is 24.6 eV compared to 15.8 eV for argon [123]. The high ionization energy enhanced the possibility of energy transfer to the analyte. A helium plasma is able to excite efficiently elements introduced into the plasma, and has low background characteristics. Hydrogen (BOC Gases, The BOC Group, Inc., Murray Hill, N.J.) was introduced into the

plasma at a flow rate of 250 cm^3/min for the cleaning step. The presence of hydrogen in the plasma helped to create a reducing environment and increased the temperature of the plasma [124]. The higher temperature and reducing environment helped in the removal of the carbonaceous residue left over from the blood sample.

Electrode

The graphite cup holder electrodes were made out of spectroscopic grade carbon (Union Carbide, Carbon Products Division, Cleveland, OH). The metals used for the cups and the electrodes were obtained from Alfa Aesar/Johnson Matthey, Ward Hill MA. The following metals were obtained as rods and machined to make the various electrodes: nickel (99.5% pure), titanium (99.99% pure), and tungsten (99.95% pure). The tungsten screen used was obtained from Newark Wire Cloth Co., Newark, NJ.

The tungsten wire (99.95% pure) used was also obtained from Alfa Aesar/Johnson Matthey, Ward Hill, MA. Three diameters of wire were used: 0.25 mm, 0.5 mm, and 0.75 mm. The final filament used was made out of the 0.5 mm tungsten wire. The top of the filament was a tight 2.5 turn spiral with a diameter of 3 mm. The total length of the filament was 6.5 cm.

Lens Setup

The initial lens setup (figure 4-3a) used two planoconvex lenses. The first lens (diameter = 50.8 mm, focal length = 125 mm) was placed 125 mm from the plasma to collimate the emission from the plasma. The second lens (diameter = 25.4 mm, focal length = 50.8 mm) was placed so that the emission was focused onto the entrance slit of the spectrometer.

In an attempt to improve the precision of the CCMP-AES, the lens setup was changed after the lead-in-blood work was completed. The lenses (figure 4-3b) were set up so that the emission from the plasma filled the collimating mirror of the spectrometer. Two lenses had to be used because a single lens could not be placed close enough to the plasma for the desired focusing. A first lens (focal length = 38.1 mm, diameter = 38.1 mm, Esco Products/Precision, Oak Ridge, New Jersey) was used to form a one-to-one image of the plasma at a proper distance away from the plasma. It is placed 76 mm from the plasma. The second lens (focal length = 25.4 mm, diameter = 25.4 mm, Esco Products/Precision, Oak Ridge, New Jersey) was then used to magnify the image in such a way that the collimating mirror of the spectrometer was completely filled with emission. The second lens was placed 102.78 mm from the first lens. The distance for each

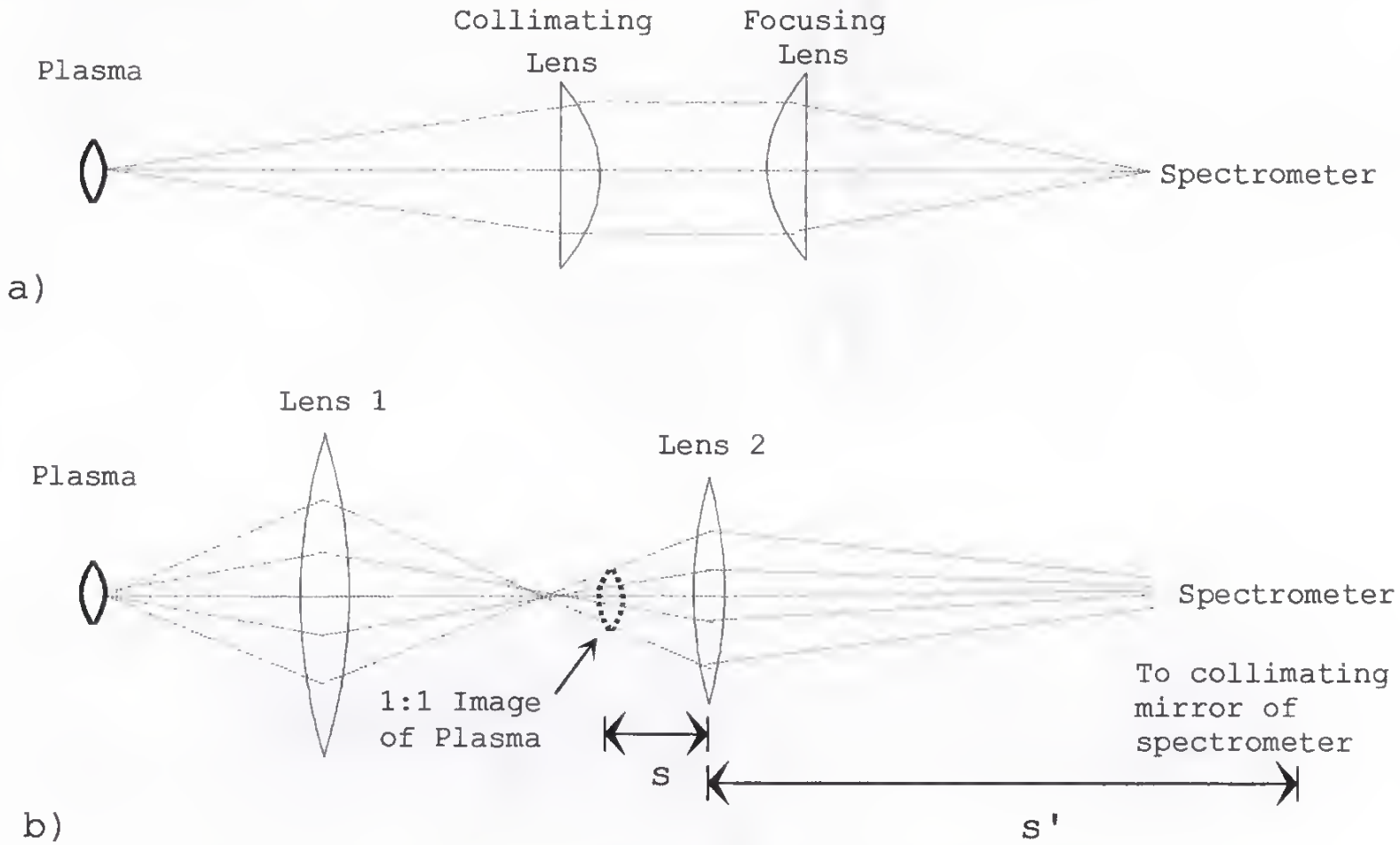


Figure 4-3. Lens setup (not to scale): a) lead in blood work, b) multielement work.

lens was calculated from the equations:

$$1/f = 1/s + 1/s' \quad \text{and} \quad m = s'/s$$

where f is the focal length of the final lens, s is the distance between the emission source and the final lens, s' is the distance between the lens and the mirror, and m is the resulting magnification of the image. In the modification of this lens setup, s became the distance from the second lens to the one-to-one image formed by the first lens. This lens setup resulted in a magnification of the plasma image of approximately 25 times.

Detector

The detector consisted of a 0.5 m spectrometer (Spex 1870, Edison, NJ, USA) and either a photodiode array (PDA) or a charge coupled device (CCD). The spectrometer grating contained 1200 grooves/mm with a blaze wavelength of 300 nm. The preliminary work and the lead in blood research was done using the PDA. The multielement work was done with the CCD.

The spectrometer slit width was adjusted for each element. If greater sensitivity was needed, the slit width was opened to as much as 40 μm. For elements requiring less sensitivity and higher resolution, a slit width as small as 10 μm was used. The slit height was kept constant at 2 cm. Both the PDA and the CCD gave a spectral window of 40 nm.

Photodiode array

The intensified photodiode array (Tracor Northern TN-6122A, Middleton, WI, USA) consisted of 1024 silicon photodiodes arranged linearly, each spaced 25.4 μm apart. Each photodiode consisted of a layer of silicon doped with atoms containing extra electrons (p-type semiconductors) on top of a layer of silicon doped with atoms with one valence electron less than silicon (n-type semiconductor). This allows the current to flow in only one direction. A reverse biased potential is applied across the diode so that when exposed to light, electron hole pairs are created producing a current that is proportional to the amount of light [125-126].

Charge coupled device

The detector was changed from the photodiode array (PDA) that was used for much of the lead-in-blood work, to a charge coupled device (CCD) for all of the multielement work. This change was necessary because of problems that developed with the hardware and software that controlled the PDA. The CCD detector has the advantage that it was two dimensional and was cryogenically cooled to reduce the dark current.

The CCD contained 296 x 1152 picture elements (pixels). Each pixel was 20 μm square and consisted of a metal-oxide-silicon (MOS) capacitor. The pixels were made out of an

insulating silicon dioxide layer over a p-type silicon substrate. This was topped by a thin metal electrode [125, 127-128]. When a photon struck a pixel, it penetrated the lattice breaking the covalent bonds between adjacent silicon atoms. This created electron-hole pairs which were measured as an electric charge. The radiation striking each pixel was proportional to the resulting charge and was measured by transferring the charge to a single point. The covalent bonds could also be broken by thermal agitation. The thermal generation of charge was reduced by cooling the CCD. Figure 4-4 shows the effect of cooling on the CCD background counts. The temperature was maintained constant by a heating element in the CCD dewar. The temperature was maintained at -110 °C even though there was not much change in the dark counts below a temperature of -40 °C. At temperatures higher than -90 °C the liquid nitrogen evaporates too quickly. At temperatures lower than -140 °C, the charge transfer efficiency from pixel to pixel may be lowered, degrading the CCD performance [126].

If the light levels reaching the CCD were too high, blooming could occur. Blooming is the spillage of charge from an over-illuminated pixel to an adjacent pixel [126, 128]. The signal to noise ratio and the dynamic range could be improved by the process of binning. Binning combines the charge from adjacent pixels during readout. The charge read

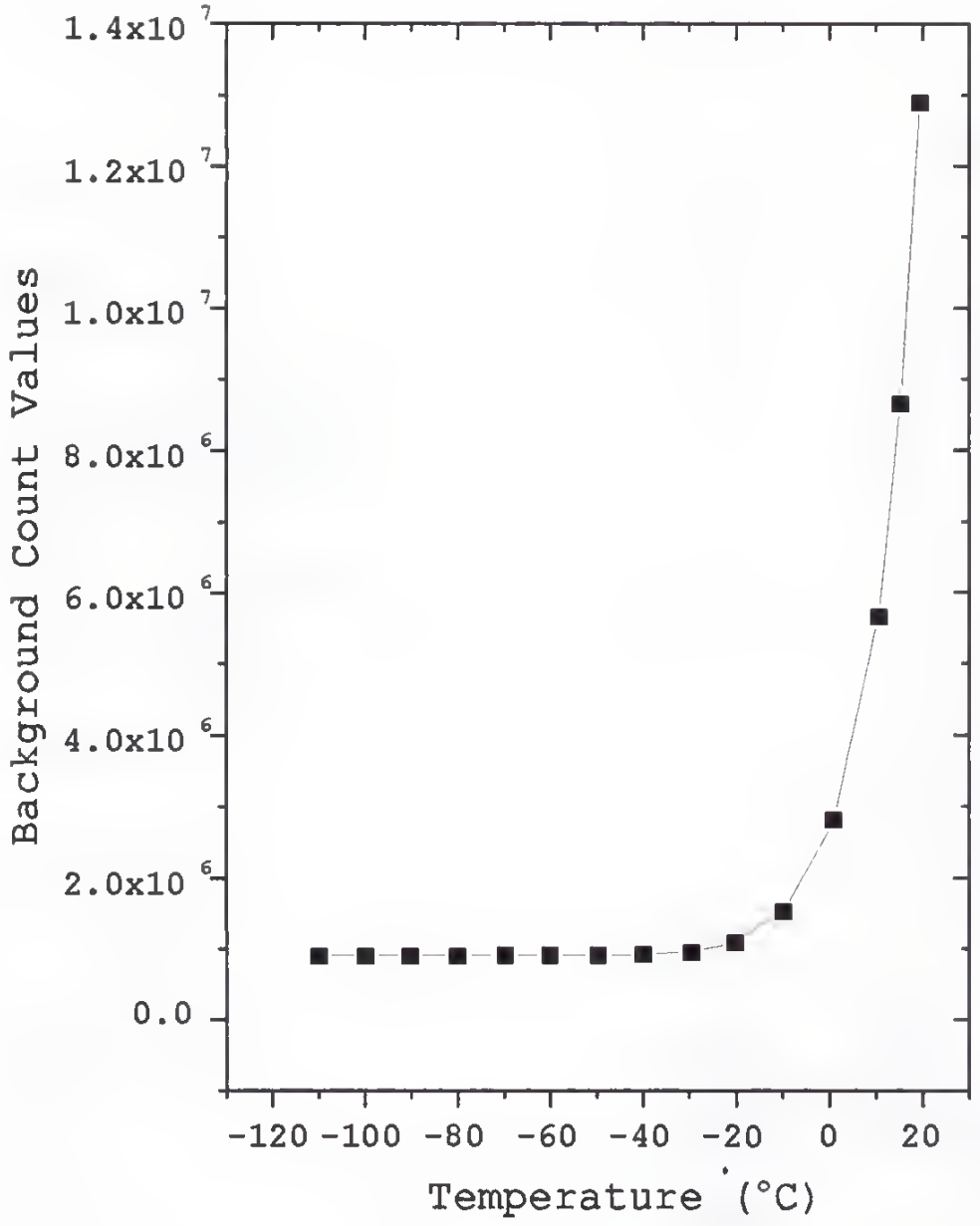


Figure 4-4. CCD background dependence on temperature.

will increase by the number of pixels binned, but the noise will stay the same. Binning has the disadvantage of reducing the spatial resolution [126].

Originally, the CCD detector was not sensitive to emission below 400 nm. The camera was sent to Spectral Instruments (Tucson, Arizona) so that a UV enhancement coating could be applied to the CCD element. The coating was lumogen yellow, an organic phosphor. The phosphor absorbs light in the UV range and re-emits it in the visible range.

Computer Software

The programmable power supply and the triggering of the detector were controlled by a computer (PC's Limited, model 28608L, PC's Limited, Austin, TX) and a computer interface (Model SR 245, Stanford Research Systems, Palo Alto, CA, USA) using a program written in Microsoft QuikBasic 4.50 (Copyright Microsoft Corporation, 1985).

The emission spectra were collected using CCD9000™ spectral acquisition software, version 2.2.2 (copyright 1990-1992, Photometrics, Ltd.). The peak areas were determined using the program LabCalc™ (copyright 1987-1992, Galactic Industries Corporation). Analytical curves were constructed using Origin™ version 4.0 (copyright 1995, Microcal™ Software, Inc.).

Materials

Aqueous Standards

Aqueous standards were prepared by sequentially diluting 1000 ppm reference standards for each element (Fisher Chemical, Fisher Scientific, Fair Lawn, New Jersey). The standards used in the standard additions of the blood analysis were prepared from the salts of the elements being analyzed. This was necessary because the standards needed to be non-acidic to prevent denaturing of the blood. Also, the concentrations required for some of the elements were larger than the available aqueous standards. All the aqueous standards were prepared using deionized water (specific resistivity 18 MΩ/cm) from a Milli-Q Plus water system (Millipore Corporation, Bedford, MA). The aqueous standards were introduced for analysis using a 2 μL air displacement pipetter (Eppendorf, Brinkman Instruments Inc., Westbury, NY).

Blood Standards

The lead bovine blood standards used were Quality Control Materials (QCM) produced and distributed by the CDC Blood Lead Laboratory Reference System (BLLRS). The samples were collected by the CDC from two cows kept at the CDC livestock facility (Lawrenceville, GA) that were given

dosages of lead nitrate in gelatin capsules. The blood was collected from the cows and the initial concentration was determined using atomic absorption spectrometry. Varying amounts of the two blood samples were then blended to give a range of lead concentrations. The final concentrations of the samples were determined using ID-ICP-MS [70].

Human whole blood was collected by venipuncture into a Vacutainer (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) coated with KEDTA as an anticoagulant. The standard addition samples were made by adding varying amounts of an aqueous standard to a 0.75 mL portion of whole blood. Deionized water was added to the sample to produce a final volume of 1.0 mL. This resulted in a sample that was 75% whole blood. The samples were gently rolled and then sonicated for 5 minutes to thoroughly mix the aqueous standard and the blood.

A lead-in-blood Standard Reference Material (SRM 955a) was purchased from the National Institute of Standards and Technology (NIST) (Gaithersburg, MD). This SRM consisted of four vials of frozen bovine blood each containing a different concentration of lead. The concentration of lead in each SRM was determined by NIST using ID-ICP-MS and confirmed using GFAAS and laser-excited atomic fluorescence spectrometry. The concentrations are shown in Table 4-1. The uncertainty reflects a confidence level of 95%.

It was necessary to keep the blood samples frozen when not in use. By freezing the blood, the bacterial and chemical interaction of the blood sample were greatly reduced. If not frozen, the various elements can bind to proteins in the blood and settle out. Although the content of the element in the vial remains the same, the concentration in the liquid portion will be less than the target value for the standard. The proteins could also denature leading to a change in the homogeneity of the blood [129]. Prior to use, the blood samples were allowed to thaw to room temperature, homogenized by gently rolling, and then sonicated for 10 minutes.

The blood samples were introduced for analysis by a positive displacement micropipetter (Drummond® model 525, Drummond Scientific Co., Broomall, PA). Before depositing the sample, the outside of the glass capillary tip was wiped with a Kimwipe™ to remove any blood that had adhered to the tip. The pipetter was cleaned between sample runs by repeatedly depressing the plunger first in a solution of 5% nitric acid solution and then in deionized water.

Table 4-1. Concentration of lead in SRM 955a at 22°C.

Vial Number	Concentration (ppb)
955a-1	50.1 ± 0.90
955a-2	135.3 ± 1.3
955a-3	306.3 ± 3.2
955a-4	544.3 ± 3.8

CHAPTER 5
SAMPLE INTRODUCTION

Introduction

Capacitively coupled microwave plasma atomic emission spectrometry (CMP-AES) has been used to analyze various types of samples directly. Discrete sample introduction in a CMP is easier than in a microwave induced plasma or an inductively coupled plasma because a CMP is the only one that uses an electrode to support the plasma. Investigations have involved the analysis of various matrices including dry tomato leaf samples, coal fly ash, steel, oil, and biological materials [1-3, 130-132]. A variety of methods have been used for sample introduction into a CMP. They have included nebulization, thermal vaporization, and hydride generation.

Methods of Sample Introduction into a CMP

In the earliest work with a CMP as an atomic emission source, solid electrodes were used to support the plasma. The analyte solution was vaporized and carried into the plasma by premixing the analyte carrier gas and the plasma

gas. Hanamura *et al.* used a platinum clad tungsten electrode with this method of sample introduction [133]. The platinum coating was used because the platinum is thermally stable, chemically inert, and has a low thermionic emission rate. These properties of platinum increased the electrode lifetime and reduced the contamination of the plasma by elements present in the electrode. Interfering emission lines from the electrode is one of the major drawbacks of the single electrode CMP. Hanamura and coworkers use this type of electrode to analyze hydrogen and oxygen in metals and also mercury in water [133-134].

Nebulization

Several researchers have used a nebulizer to introduce aqueous samples into a CMP [124, 135-137]. A nebulizer is an easy and inexpensive way to introduce a solution into a plasma. Nebulization is a process where the sample to be analyzed is transferred by a peristaltic pump to a nebulizer which converts the sample into an aerosol in a spray chamber. The aerosol is then swept by a carrier gas through the center of the electrode into the plasma. A disadvantage of nebulization is that much of the sample is lost in the spray chamber.

Patel *et al.* used pneumatic nebulization with a CMP to analyze aqueous samples for 15 elements [135]. Sample solutions were nebulized with a Meinhard nebulizer and a laboratory-constructed spray chamber and desolvation system. A tubular electrode made out of tantalum was used to support the plasma. The analyte carrier gas passed through the center of the hollow electrode and entered into the plasma at the top of the electrode [25]. By introducing the sample directly into the core of the plasma, the interactions between the sample and plasma were improved. Also, the concentration of the analyte in the plasma viewing region is increased, improving the detection limits, signal to noise, and signal to background. It was determined that this method gave low detection limits with a wide linear dynamic range for a number of different elements. Several other tube materials and forms of electrodes were evaluated. The electrode types included a platinum tube, a copper tube, a platinum coated tungsten wire (0.5 mm o.d.) a molybdenum rod, and a tungsten rod with platinum cladding [135].

Hwang *et al.* used graphite as the electrode material [137]. This electrode had a lower emission background and did not significantly contaminate the plasma in comparison to the metal rod electrode. Excellent detection limits for several elements in aqueous solutions were obtained [137].

Thermal Vaporization

Two previous methods of sample introduction by thermal vaporization (TV) include a tungsten filament electrode (figure 5-1a) [23, 131-132] and a cup holder electrode (figure 5-1b) [1-3, 130, 138-139]. The sample was introduced into the CMP by directly depositing it on the filament or in the cup held by the electrode. Thermal vaporization is advantageous over nebulization in that a greater percentage of the sample is introduced into the plasma. TV has the disadvantages of poorer precision, greater interference effects, and a lower throughput of samples.

Hanamura *et al.* used a another method of thermal vaporization with a CMP to measure carbon, hydrogen, nitrogen, oxygen and mercury in orchard leaves and tuna fish [140]. A separate furnace vaporizer was used. The sample was held in a quartz crucible which was heated. The carrier gas was flown through the sample chamber to carry the volatile constituents through the center tube of the torch and into the plasma for analysis.

Cup electrode

A cup can be used to introduce both liquid and solid samples into a CMP. In order to use a cup electrode, an electrode must be fabricated such that the top of the

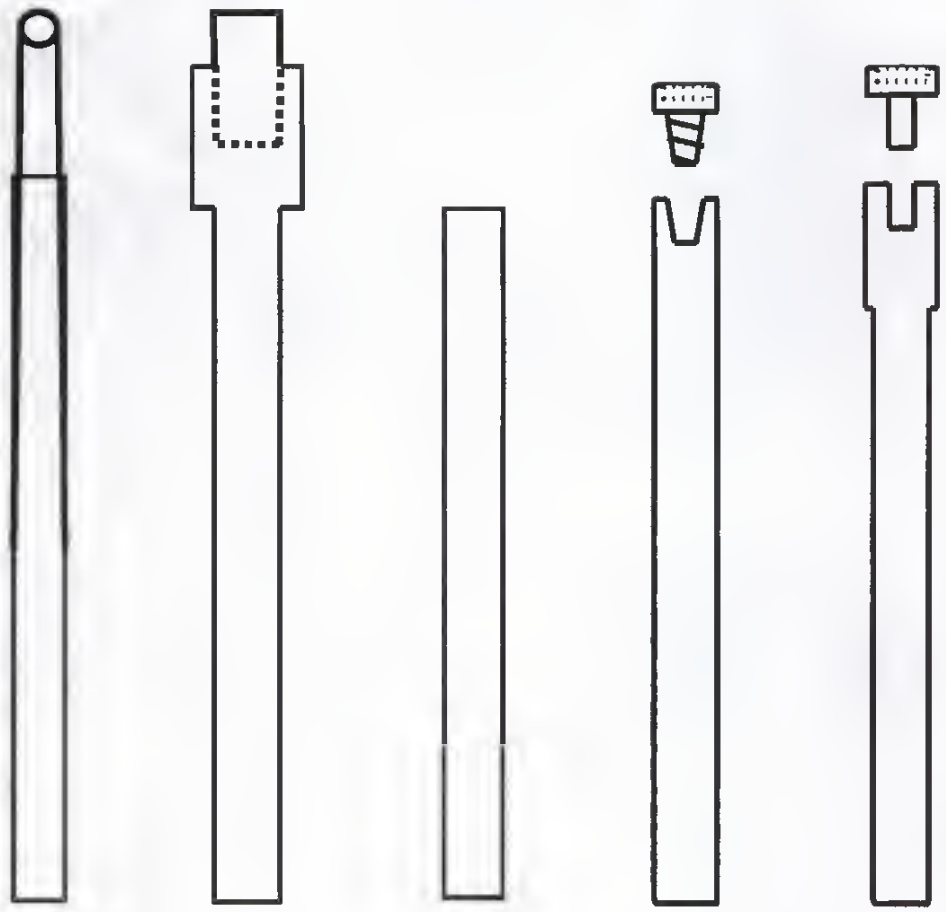


Figure 5-1. Electrode designs: a) filament electrode, b) cup holder electrode, c) platform electrode, d) titanium electrode with nickel cap, e) titanium electrode with titanium cap.

electrode has a hollowed out portion that will snugly hold the cup. The electrode must be made out of a material that is conductive and has a higher melting point than the thermal temperature of the plasma. Materials that can be used for the electrode are graphite or various metals. Graphite electrodes are cheaper and more resistive than metal electrodes, and emission from the metal electrode can also cause interference in analysis. Electrodes made out of metal have several advantages over those made out of graphite. Metal electrodes are more durable and last longer than graphite electrodes. Also, graphite electrodes form refractory carbides and produce gaseous molecular carbon species which cause interference in emission measurements.

Cups made out of both graphite and tungsten have been used to hold the sample. The cup was placed on the top of the electrode and the electrode containing the cup was placed into the central tube of the torch. The plasma was ignited at a low power (around 100 W) to ash the sample and was then raised to 400 - 700 W to atomize and excite the analyte, enabling the measurement of the emission [1]. By using a cup instead of a wire filament, higher powers could be used so that there were fewer matrix effects and the signal was larger. The disadvantage of using a cup was that the atoms were dispersed over a wider volume so the number density of excited atoms was smaller.

Ali *et al.* used CMP-AES with a cup electrode with both the electrode and the cup made out of graphite. Detection limits ranging between 10 and 210 pg were obtained for 12 elements with a precision better than 12% [138]. A sample volume of only 5 μL was used. The graphite cup was coated with tantalum carbide to reduce memory effects. Because graphite was quite porous, memory effects were observed for all the elements analyzed. The cups lasted 30-40 firings and then had to be replaced due to etching of the cup rim by the plasma. Multielement analysis with this system was performed on coal fly ash and tomato leaves [1]. Spencer *et al.* used a tungsten cup to analyze silicon in oils [3]. Tungsten was found to be an excellent cup material because of its tolerance to high temperatures, long lifetime, low emission background, and low memory effects.

More recently Pless *et al.* used a tungsten cup in a graphite electrode for multielement analysis [139] . The cup had a total volume of 30 μL. Detection limits in the low picogram range were obtained for 10 μL samples of cadmium, magnesium, and zinc in aqueous solutions. Cadmium in solids was also analyzed obtaining a detection limit in the picogram range [130]. Various matrices were analyzed by this system including coal fly ash, tomato leaves, soil, bovine liver, and oyster tissue. The results achieved good

agreement with the certified values of the reference materials.

Filament electrode

Ali and Winefordner evaluated a tungsten filament electrode for multielement analysis in aqueous solutions [23]. Filaments have the advantage that they are simple and inexpensive. The sample was introduced into the plasma by placing a few microliters of sample in a loop in the filament. The sample was then dried at low microwave power. The filament heated up rapidly creating a high rate of volatilization. After the sample was dry, the plasma was ignited and the sample was ashed if necessary by a low power (30 W) microwave plasma . The power of the plasma was increased until the sample was atomized and excited so that the emission could be measured. It was found that adding a low flow rate (100 mL/min) of hydrogen gas with the plasma gas reduced the background emission from the tungsten filament. The absolute detection limits of 12 elements were in the range of 1 to 100 pg and this compared favorably to the method of graphite furnace atomic absorption spectrometry (GFAAS). A linear dynamic range of 3 to 4 orders of magnitude was obtained and the precision was better than 10%. Reported lifetimes for the filaments were 500-1000 runs [23].

Wensing *et al.* evaluated a CMP-AES for a lead blood screening method using a tungsten loop (figure 5-1a) as the electrode [131-132]. A tungsten wire of 0.25 mm thickness was tied in a knot, leaving a small loop in the center, and the remaining ends of the wire were bent so that they could be inserted into a piece of quartz tubing which was then inserted into the plasma torch. The blood samples were held in the loop by adhesion to the wire.

A 5 µL blood sample was placed in the filament loop and subsequently dried, ashed, and atomized. Drying was accomplished using microwave power to inductively heat the electrode for 90 seconds. After drying, the helium gas flow was turned on and a small plasma was ignited, ashing the sample at a power of 55 W for two minutes. The sample was then atomized in a helium plasma at a power of 170 W. The lead emission at 405.8 nm was measured using a photodiode array (PDA). A cleaning step was necessary in order to remove the carbonaceous residue from the left over blood sample. Cleaning was performed by increasing the power to 200 W and adding a flow of hydrogen gas to the helium plasma. The cleaning procedure lasted for one minute and effectively removed all blood residue from the filament.

The filament electrode CMP-AES method was able to meet two of the criteria set forth by the Centers for Disease

Control (CDC) for a lead in blood screening method. The detection limit for lead in blood was 7 ppb and the analysis time was under five minutes. However, the filament electrode was not accurate for blood lead concentrations unless matrix effects were reduced by diluting the blood with deionized water by a factor of approximately one half. Even with dilution, the method was not sufficiently accurate for blood lead concentrations below approximately 200 ppb and the precision did not meet the requirements set forth by the CDC for lead in blood screening methods.

Using the filament as the electrode had several drawbacks. The filaments were handmade and so were difficult to make reproducibly. It was also difficult to deposit the sample in the filament loop with adhesion to the wire as the only source of support for the sample. Finally, the lifetime of the filament electrode was greatly shortened if the microwave plasma power was raised above a certain point.

Hydride Generation

Hydride generation involved introducing the elemental analytes to the CMP as a gas. An acidified aqueous solution of the sample was added to a small volume of 1% sodium borohydride in a reaction cell. After a certain amount of time had passed, the resulting hydride of the element was carried to the CMP by a flow of the plasma gas. Akatsuka

and Atsuya used a CMP with hydride generation to analyze arsenic in sewage sludge, and iron in steels. They obtained a detection limit of 0.25 ppb for arsenic in solution [141]. Uchida *et al.* used the method of hydride generation with a CMP to analyze inorganic tin [142].

Development of Electrode for Blood Analysis

Cup Holder Electrode

A cup holder electrode (figure 5-1b) was investigated for the analysis of lead in blood. A cup holder electrode had several advantages over the filament electrode used by Wensing *et al.* for lead blood analysis. Using a cup holder electrode allowed the introduction of larger sample volumes and made sample deposition easier and more reproducible. Also, a cup holder electrode is a more robust electrode allowing the atomization power to be increased, which could lead to increased emission intensity.

Initially the electrode material chosen was graphite. Graphite is a good material because it can sustain very high temperatures, it is inexpensive, and it is easy to machine to make modifications to the electrode. The easy machinability of graphite allowed various parameters of the electrode (length and penetration) to be optimized before

switching to a metal cup holder. The metal holder would be more durable but not as easy to machine as graphite.

The graphite electrode contained a hole in the top which held a nickel cup. A graphite cup would not be suitable for blood analysis because the graphite could form refractory compounds which would interfere with the lead signal. Also, the blood could seep into the graphite causing memory effects. A cup made out of metal would be a better choice because there would be less of a chance of interfering species and memory effects. Initially, nickel was chosen as the cup material because it did not oxidize easily, making it very durable. The cup had a sample capacity of 20 μL.

Several parameters for the cup electrode were studied. The change in electrode from the filament to the cup electrode required reoptimizing the conditions of the plasma. The coupling of the microwave energy and the stability of the plasma were affected by the length of the electrode, and the electrode's penetration into the waveguide. The optimum coupling position of the electrode was determined by varying the electrode length penetration into the microwave field to find the parameters where the minimum microwave power was needed to sustain the plasma.

The first parameter studied was the length of the electrode. Initially the height of the electrode above the

waveguide was kept constant at 8 mm. This was the height used in the work with the tungsten filament electrode. Different lengths of the electrode were then used determining the range of powers over which a stable plasma would form. The power supplied to the magnetron was gradually increased until a plasma would form. The power was increased until the plasma was no longer stable and then decreased until the plasma could no longer be sustained. The lengths of the electrodes ranged from 3.5 cm to 7.0 cm which corresponded to a depth of penetration into the waveguide of 2.7 cm to 5.7 cm. This experiment was repeated keeping the penetration depth of the electrode into the waveguide constant at 3.2 cm and evaluating different lengths of the electrodes. As the penetration depth was increased, the plasma could be maintained at higher powers. For some positions of the electrode, the maximum power of 700 W could not be achieved because the plasma started to make a very loud whining noise. The length and penetration that produced a stable plasma over the widest range of powers was chosen as the optimum. Figure 5-2 shows some examples of the data collected for the optimization of electrode length and penetration.

Using the optimum conditions found for the electrode, the analysis of aqueous lead solutions was studied. At an atomization power of 370 W, the lead emission took

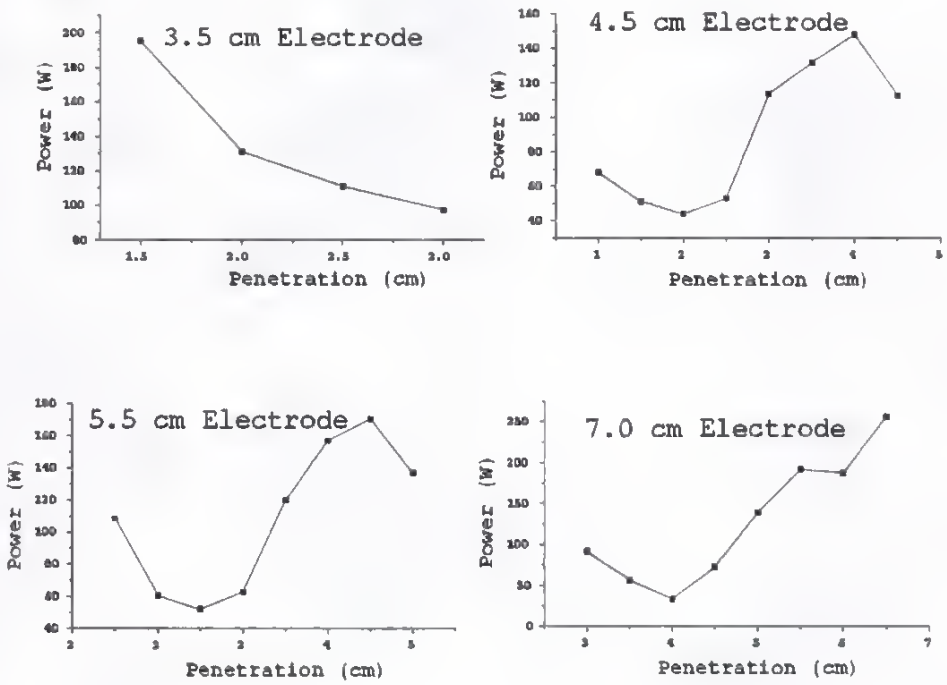


Figure 5-2. Optimization of electrode length and penetration depth of graphite electrode.

approximately 20 seconds to appear and then remained for 90 seconds for a 10 μL sample of a 50 ppm lead standard. At such a high concentration and power, the signal should have been much greater. Tungsten and titanium cups were evaluated with the graphite cup holder, but these cups also gave poor results.

The next electrode evaluated was a cup holder made out of titanium with a nickel cup. Titanium metal instead of graphite could improve the coupling of the microwaves to the electrode, improving the efficiency of atomization and excitation. After atomization with this system, the cup remained very hot and required a long time to cool down. If an aqueous sample was deposited in the cup before it was sufficiently cooled, the sample would vaporize. No improvement of the lead signal intensity was observed.

Platform Electrode

A cup holder electrode made out of titanium that had not been drilled to hold a cup was then studied as the method of sample introduction. This type of electrode was labelled the platform electrode because the sample was placed on the flat top portion of the electrode. The top portion was approximatly 6.5 mm long and 6 mm in diameter and the post was ~ 3 mm in diameter. A 10 µL aqueous lead sample was placed on the flat top of the electrode, dried

for 90 seconds at approximately 150 W and then atomized with a plasma power of 300 W. The titanium platform electrode gave a larger signal than had previously been obtained. The signal still lasted a long time (figure 5-3a) but not as long as with the graphite electrode and the nickel cup. The signal increased (figure 5-3b) when the bulky top part of the electrode (that was intended to hold the cup) was removed, yielding a thin metal rod (Figure 5-2c). With this electrode design, the plasma formed on the same surface that the sample was on, increasing the interaction of the sample and the plasma, yielding more efficient atomization and excitation.

A significant problem was experienced with the titanium electrode. After several runs, the lead signal would begin to decrease in intensity and take longer to appear. It was necessary to sand off the top of the electrode to regain the larger signals. Each time the electrode was sanded, a titanium atomic emission line appeared that interfered with the lead line being used for measurement. The electrode had to be tempered by igniting a plasma and then slowly taking it to higher powers to eliminate the interference before conducting another run after cleaning. The more the electrode was used, the sooner in between runs it had to be cleaned off. The interfering titanium line also limited the amount of power that could be applied to the plasma. At

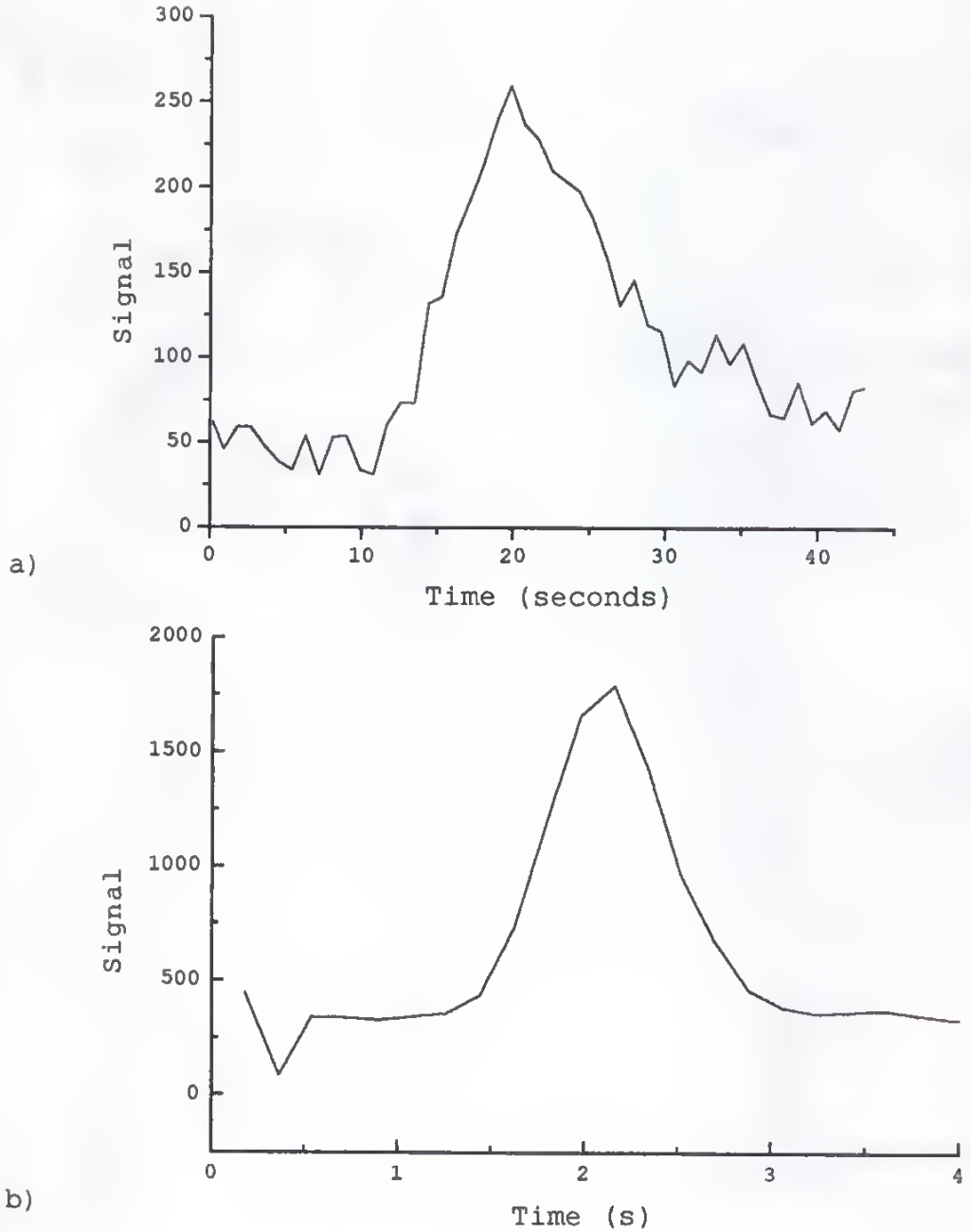


Figure 5-3. Temporal profiles for lead signal for the platform electrodes: a) platform with cup holder portion, b) thin titanium rod platform.

powers above 400 W, the interfering line appeared. It is desirable to have an electrode that could last for an indefinite period of time and would not have any emission lines which would limit the powers used. For these reasons, several other materials were tried for the platform electrode.

Tungsten, which had been used for the filament electrode, has a higher melting point and lower background emission than titanium and so it was evaluated as the electrode material. A 10 μL aqueous sample on the tungsten electrode took approximately 5 minutes to dry at a microwave power of 150 W. The lead signal, upon atomization, took several seconds to appear and then lasted for about 30 seconds. The signal was small compared to the signal obtained using the titanium electrode, even at higher powers. Nickel was studied next, but it also produced results similar to those obtained with the tungsten electrode.

A titanium electrode with a nickel cap which screwed into the top was then evaluated (figure 5-2d). This design was used in an attempt to obtain a similar signal as that obtained with the titanium electrode, but with a longer lifetime because of the nickel cap. The signal obtained for this electrode was similar to that obtained when the whole

electrode was nickel; a low intensity signal was delayed in appearing and had a long temporal profile.

From the results obtained, the electrode made out of pure titanium was the best platform electrode even though it would have to be changed on a regular basis. The titanium platform electrode lasted approximately 130 firings for aqueous samples. The titanium platform gave good results for aqueous lead samples (figure 5-4) achieving a detection limit of 30 ppb for a 5 µL sample volume. However, the precision was poor for concentrations of 100 ppb and below.

Analysis of lead in whole blood was performed using whole blood quality control materials (QCMs). The analytical curve for these standards (figure 5-5) was linear ($R = 0.997$) and produced a detection limit of 50 ppb. After running an individual analysis, it was necessary to clean the electrode by scraping off the remaining blood residue. This was not difficult, but added approximately one minute to the analysis time. Occasionally, the blood sample would interfere with the plasma yielding poor precision. The interference of the blood samples with the plasma might be due to a problem in depositing the sample. Since the surface of the electrode was flat, it was difficult to deposit the sample in the same way each time. The maximum

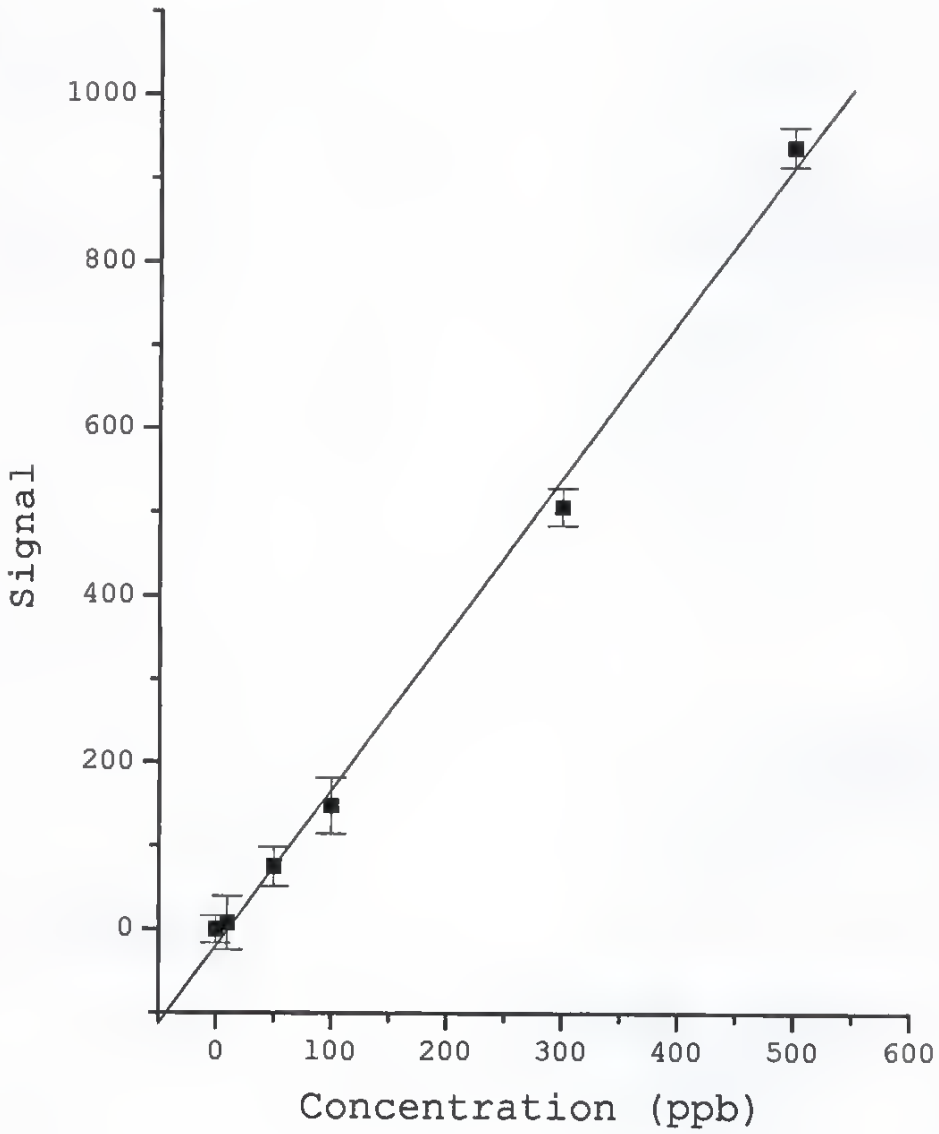


Figure 5-4. Analytical curve for aqueous lead standards on the titanium platform electrode.

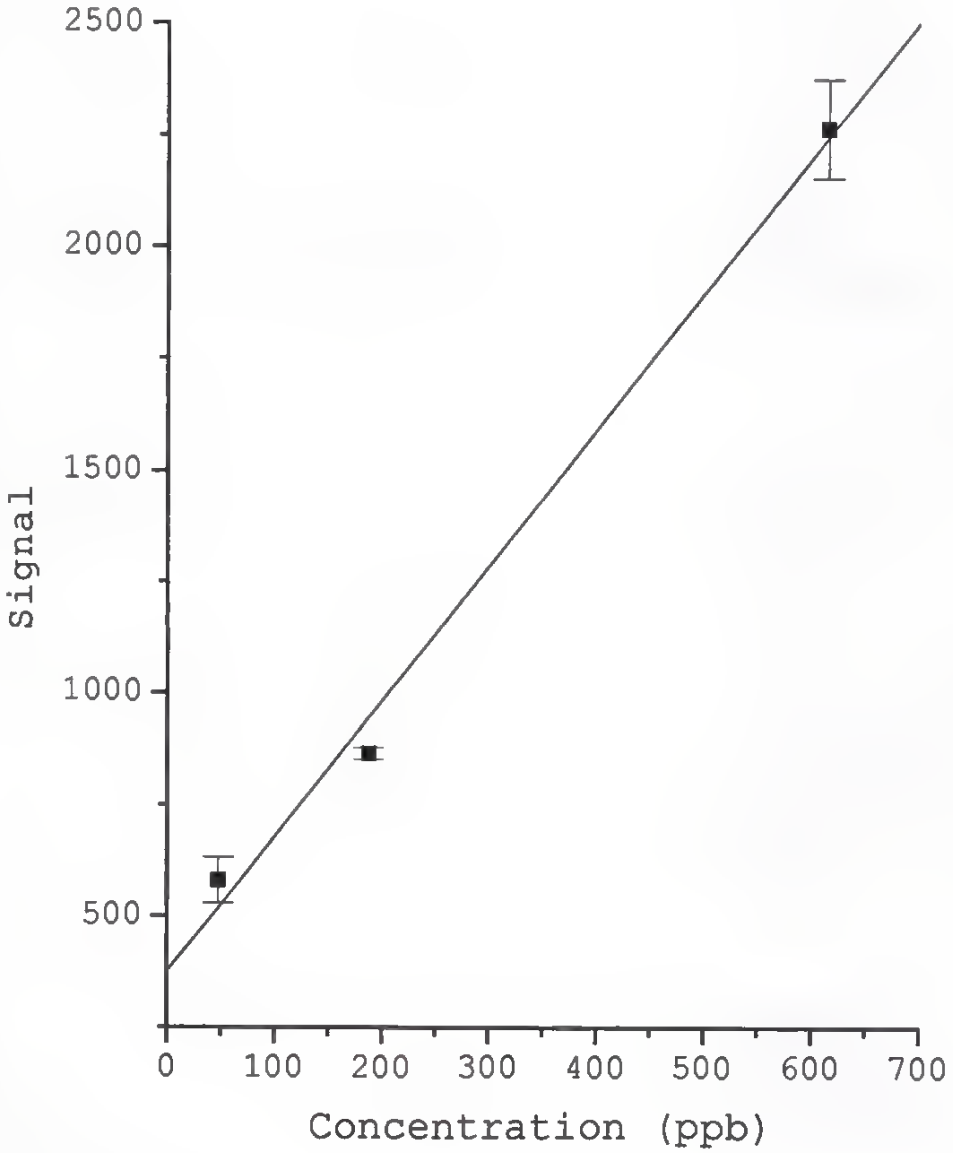


Figure 5-5. Analytical curve for whole blood lead standards on titanium platform electrode.

capacity of the titanium rod platform was 5 µL, and the sample would sometimes run over the side.

In order to achieve reproducible sample deposition, electrodes containing a depression in the top were used. It was found that the size and shape of the depression was very important in measuring the lead signal. If the depression was too deep, the signal was small. If the volume of the blood sample was too large, the blood would interfere with the formation of the plasma. When blood samples were run with the depression electrode, the inside of the depression became dirty and was difficult to clean. Even for shallow depressions, the lead signal was approximately one half of the signal obtained by the electrode without the depression. The electrode with the depression had a short lifetime of only 30 firings for blood samples.

To reduce the amount of time required to clean the electrode, a titanium electrode with a cap was used (figure 5-2e). This electrode design allowed one cap to be cleaned while another cap was being used for analysis. Samples could also be dried separately, and then placed on the cap holder to use the microwave plasma for the ashing and analysis. This shortened the analysis time by ninety seconds and could be beneficial for the storage and transport of the blood samples in the clinical setting. Caps with various diameters and various sized depressions

were used. Some problems were experienced with the uniformity of the plasma on the titanium cap electrode. At lower microwave powers, the plasma would sometimes form on one side of the cap and either stay at that side or flicker around the edge of the cap. At higher microwave powers, some of the caps yielded good signal, but it was difficult to clean them when analyzing blood samples. The caps were also difficult to reproducibly construct. Caps with the same design did not produce the same signal.

Suspension Method

The results with the platform electrode demonstrated that it is necessary for the plasma to interact directly with the blood sample. Anytime the blood sample was below the plasma in some sort of depression, the signal was drastically reduced. However, when the blood sample was on the surface of the electrode where the plasma formed, the blood would often interfere with the stability of the plasma. A method for which the sample was suspended above the electrode was used to try to account for keeping the sample in the plasma without being on the surface where the plasma forms. A titanium rod electrode was used to support the plasma and a macor holder was used to support a screen or a wire mesh above the electrode (figure 5-6). Initially stainless steel screens (10-20 and 40 mesh) were used.

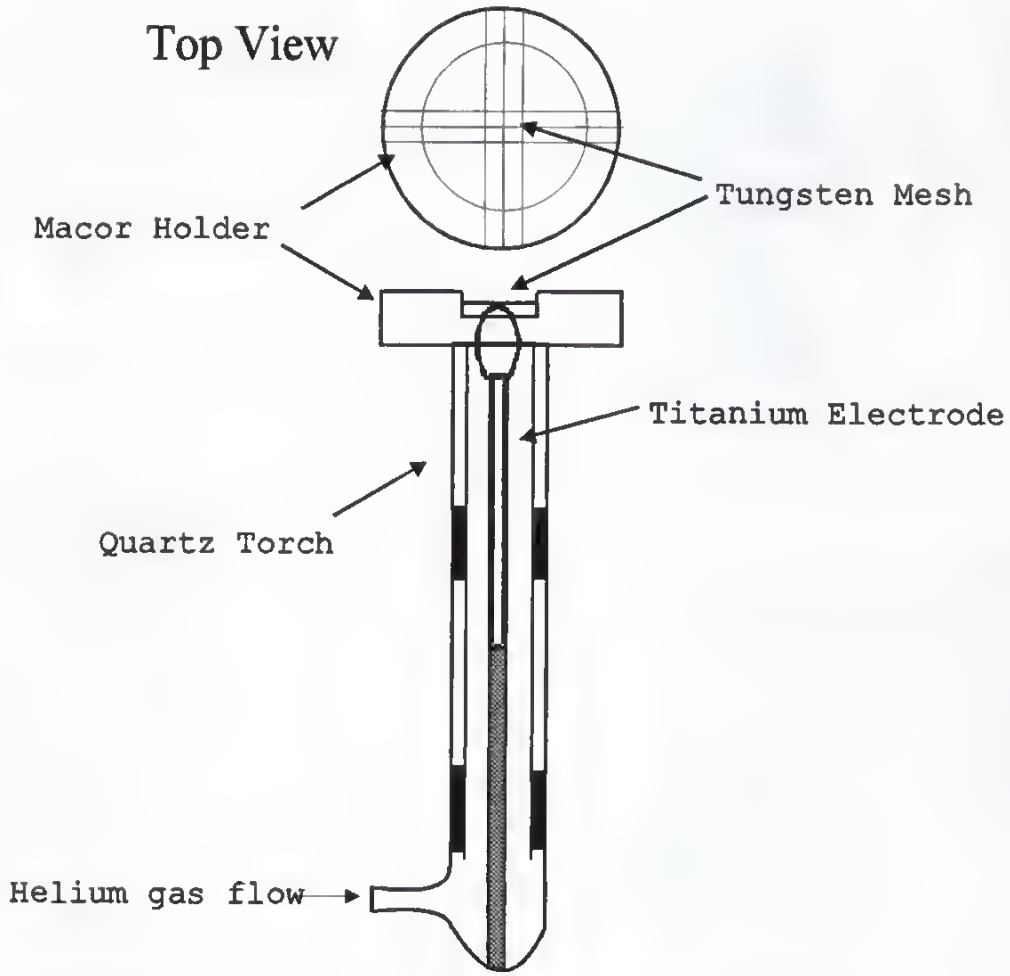


Figure 5-6. Suspension method of sample introduction.

Platinum screens (40 mesh), tungsten screens (20 and 40 mesh), and a four-squared cross made out of tungsten wire were also tried.

The sample would not dry with microwave power alone, so a very low power plasma was ignited below the screen. For blood samples, the drying caused some problems because if the plasma was too close to the sample or too high in power, it would cause the sample to bubble and spatter. The titanium electrode was changed to a pointed tungsten electrode which could sustain a very low power plasma which dried the blood more effectively. The power of the plasma was increased for ashing, and then further increased for atomization. This method worked well for aqueous standards and greatly decreased the background during atomization because the macor holder shielded most of the emission from the plasma. However, this method of sample introduction did not work well for blood samples. The mesh became very brittle at higher plasma powers and broke very easily.

Spiral Filament Electrode

Each method of sample introduction tried had various advantages to it. The cup holder electrode held the sample the best, the filament electrode was easiest to clean, the platform electrode gave the best signal, and the macor holder resulted in the lowest background. Various features

of several of these methods were combined to design an improved filament electrode. A thicker (0.5 mm in diameter) tungsten wire was used which was more durable and could sustain higher powers than the original filament (0.25 mm in diameter) could. Initially a single loop was made at the top of the wire to hold the sample, but it was difficult to deposit the sample in the loop. A two and a half turn spiral was then made at the top of the electrode The spiral served as sort of a platform and held a 2 μL blood sample very well. The spiral filament electrode (figure 5-7) performed well for both aqueous and blood samples (chapter 6) and was easy to clean. The filament was, however, difficult to make because the tungsten wire was very brittle and would often split during the construction of the spiral.

An attempt was made to use commercial tungsten light bulb filaments to hold the sample. A 20 turn, rectangular light bulb filament was placed over the loop of a filament electrode. This method would remove the necessity of having the spiral at the top of the electrode and could also help make the electrodes more reproducible. The method worked well for aqueous samples (figure 5-8) giving a detection limit of 44 ppb, but was very hard to clean after the analysis of blood samples. It also eroded quickly under the high plasma powers used to clean the blood from the electrode.

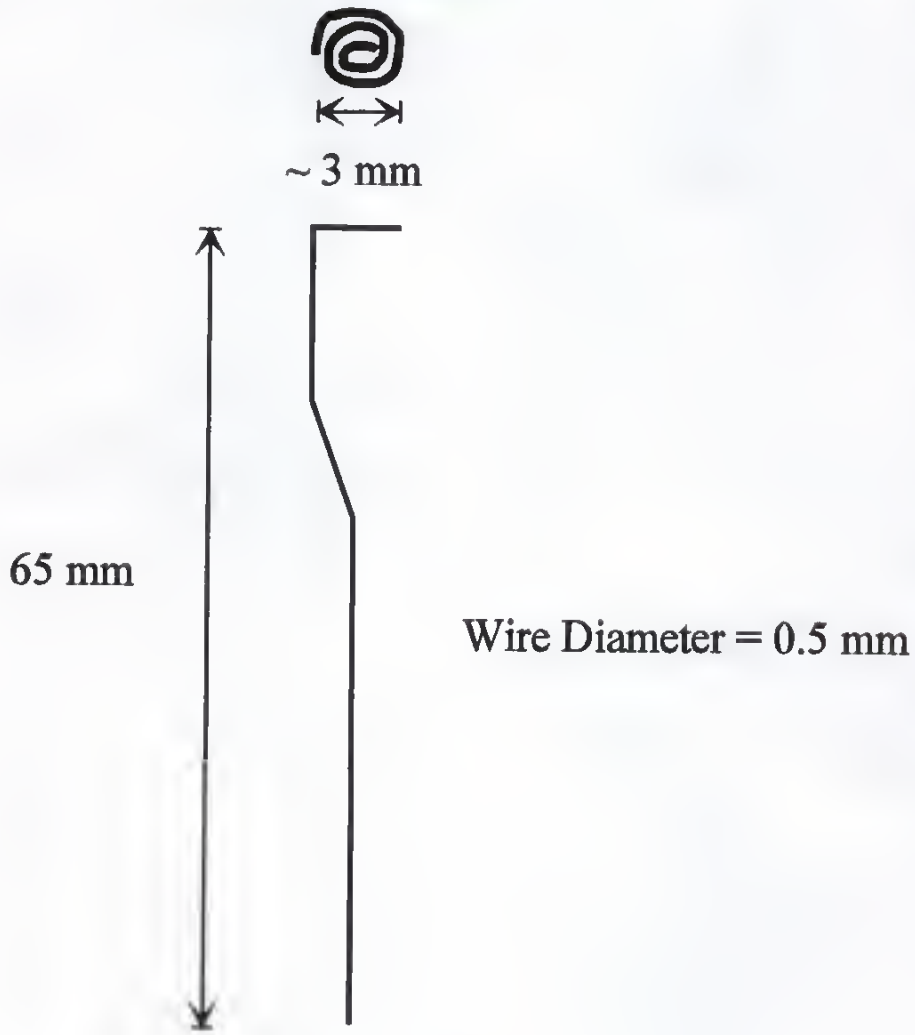


Figure 5-7. Tungsten spiral filament.

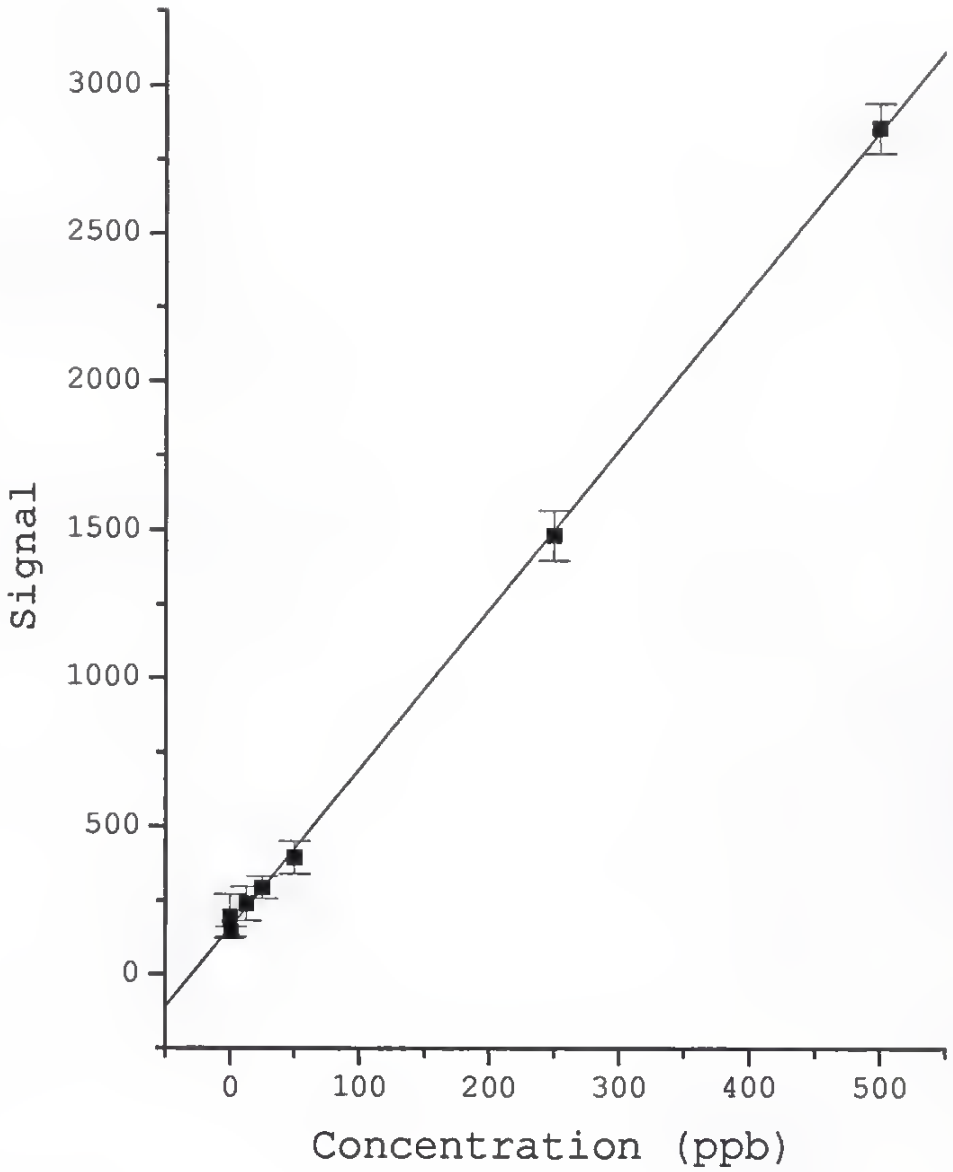


Figure 5-8. Analytical curve for aqueous lead standards on the commercial tungsten filament.

Conclusion

The best method of sample introduction was the spiral filament electrode. The spiral provided a surface which could hold the sample and support the plasma so that the sample was directly in the plasma. The sample could easily be deposited on the spiral reproducibly. The filament was also simple to clean after each analysis.

CHAPTER 6
ANALYSIS OF LEAD IN BLOOD

Introduction

The initial goal of the research was to develop the CMP-AES as a screening method for lead in whole blood. The guidelines set forth by the Centers for Disease Control (CDC) for an improved blood lead measuring system were used to evaluate if the CMP-AES was an appropriate screening method. Once the electrode design was decided, a number of other operating parameters had to be optimized.

Optimization of Parameters

Helium Flow Rate

Wensing *et al.* used a helium flow rate of 10 L/min for their work on lead in blood [131-132]. Lower gas flow rates generally increased the residence time of the analyte in the plasma, which increased the efficiency of atomization and the intensity of emission. It also reduced the amount of gas consumed. Therefore, the effect of flow rate on lead emission intensity was investigated. The emission signal gradually increased with decreasing flow rate. At flow

rates below 6 L/min, the plasma no longer appeared stable. A flow rate of 8 L/min was chosen because this flow rate produced a stable plasma and reproducible results.

Drying and Ashing Conditions

Drying and ashing steps are essential in the direct analysis of whole blood. The drying step removes the moisture content of the blood allowing a plasma to be formed on the top of the electrode. A low power was applied to the magnetron which generates microwaves in the waveguide, inductively heating the tungsten electrode. The electrode got sufficiently hot to vaporize most of the moisture in the blood. A sufficient power had to be applied to remove the moisture without causing the blood to spatter. For use as a screening method, the amount of time per analysis is very important so the drying time must not take too long.

A low power plasma was used to ash the sample. The ashing step removed much of the carbonaceous material in the blood preventing it from interfering with the emission from the analyte. Without ashing, a very high background was produced that saturated the detector. Also, the high power plasma used for atomization and emission was not stable if there was a significant amount of blood material left on the electrode.

The power of the plasma and the time used for ashing were very important. If the power was too low or the time was too short, not enough of the blood material was removed causing the problems just discussed. If the ashing power was too high or the time was too long, the analyte could be volatilized and lost, decreasing the signal. Various ashing powers (figure 6-1) and times were investigated.

The best method of ashing the blood samples involved using a number of steps, gradually increasing the ashing power. With inductive heating of the electrode, the blood sample on the electrode would never fully dry. A very low microwave power was applied and the plasma was ignited by touching a tungsten wire to the tungsten electrode. The friction from the two wires was sufficient to spark the plasma. The plasma power was then increased by a small amount causing the blood to bubble, but not spatter, until all the moisture was removed. When the blood sample was completely dry, the power of the plasma was further increased to remove much of the blood material. Two steps were used for the actual ashing of the sample, and a third step was used to ensure that the plasma formed for atomization was stable. If the plasma power jump from the ashing steps to the atomization step was too large, the resulting plasma was initially unstable.

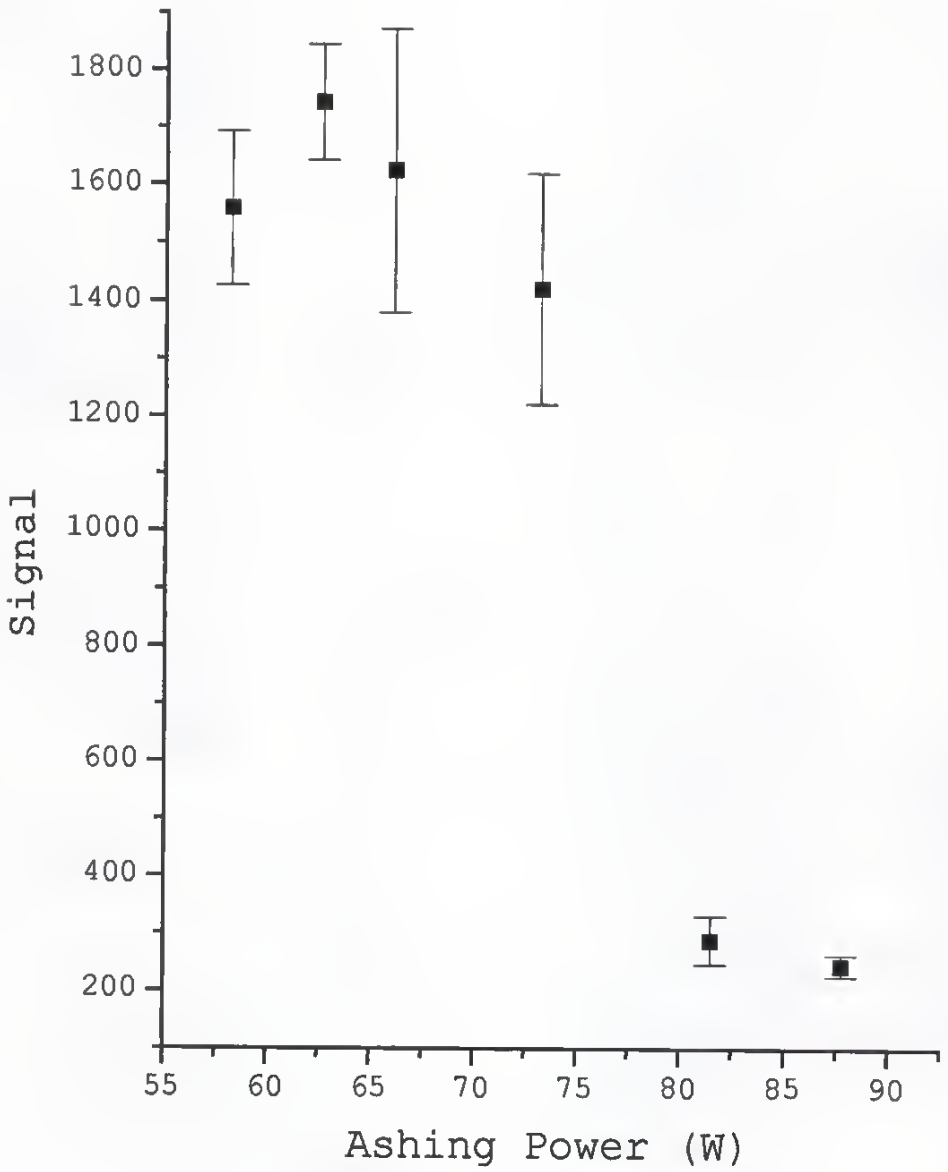


Figure 6-1. Effect of ashing power on blood lead signal.

Cleaning

The cleaning step is essential for reproducible analysis. If blood residue was left on the electrode prior to another measurement it could cause memory effects or interfere with the atomization and emission of the sample. The cleaning step must be sufficient to remove all remaining carbonaceous material without significantly reducing the lifetime of the electrode. If the cleaning conditions were too harsh, the tungsten filament became very brittle and wore away.

The cleaning procedure used involved increasing the power to 200 W with a reduction in helium flow rate to 2 L/min for 10 seconds and then a return to 8 L/min for another 10 seconds. Hydrogen gas was introduced for the cleaning step at a flow rate of 350 cm^3/min. The addition of hydrogen helped to clean the electrode by increasing the gas temperature of the plasma [124]. The decreased flow rate of helium increased the amount of oxygen in the plasma improving the oxidization and removal of the carbonaceous blood residue. After cleaning, the electrode had a very shiny appearance and was free from any carbon residue. The cleaning step reduced the lifetime of the electrode; however, it was still possible to use the electrode for close to 100 samples.

Sample Size

The sample size was very important for screening methods. One method of collecting blood samples for lead screening was the finger stick method. This involved piercing an individuals finger and then collecting blood using a capillary tube, which collected only microliter volumes; therefore, the method used must not require a sample larger than the amount collected. Larger samples are beneficial because they contain more analyte and are easier to handle and to introduce reproducibly than are smaller samples. Various sized blood samples were analyzed for lead using the CMP-AES. These results indicated that there was not a large increase in signal with sample size. The precision, however, degraded significantly with increased sample size. For very large samples, the spiral filament appeared to be overloaded, with blood hanging below the spiral top and adhering to the stem of the filament just below the spiral. Filaments with larger spirals which were able to accommodate larger sample sizes were tried, but did not give improved results. Increased ashing times were also tried to account for the larger amount of blood but were unsuccessful. A sample size of 2 μL was chosen because this volume gave good precision and was not difficult to reproducibly deposit on the electrode.

Sources of Noise

The largest source of noise for the CMP-AES was investigated. Sample runs were performed 10 times each for aqueous samples, blood samples, and an empty filament. The noise from the dark current and from fluctuations in the plasma with it continually running were also investigated. The dark current was the smallest source of noise. The noise from runs with an empty filament and with aqueous blanks was three times that of the dark current. Blood samples produced a level of noise ten times greater than the dark current. The results demonstrate that the instability of the emission background from the blood was the greatest source of noise in the blood analysis.

Analysis

Aqueous Standards

Aqueous analytical curves were constructed using 2 µL samples. The sample was dried for 60 s at 55 W. The power was decreased to 30 W and a flow of 8 L/min of helium was introduced. The plasma was ignited and immediately the power was increased to 165 W for atomization and emission. The lead emission was integrated over ten 0.18 second time intervals. The peak area of the lead signal at a wavelength of 405.8 nm was calculated for each time interval and the

peak areas were added over the lifetime of the lead signal to obtain a peak volume. The signal was then plotted vs. the concentration. The resulting analytical curve is shown in figure 6-2. A detection limit of 45 ppb was obtained. The correlation coefficient of the analytical curve was 0.9997 and the log-log slope was 1.04. The precision was 2% for concentrations greater than 100 ppb and 10% for concentrations lower than 100 ppb.

Bovine Blood Standards

A 2 µL blood sample was deposited onto the top of the electrode. The blood sample was dried by inductively heating the electrode using microwave power. After the blood sample was dry, a low power plasma was ignited and the sample was ashed in stages. The power was then increased for atomization. When atomization was complete, the power was further increased for cleaning. The steps for drying, ashing, atomization and cleaning are shown in table 6-1. The signal (figure 6-3) was analyzed by the method used for aqueous standards. Figure 6-4 shows the temporal profile for a 2 μL 411 ppb lead blood standard. Each blood standard was analyzed six times. The analytical curve obtained by running the CDC bovine blood standards is shown in figure 6-5. The error bars represent a 90% confidence interval. The relative standard deviation (RSD) for blood lead

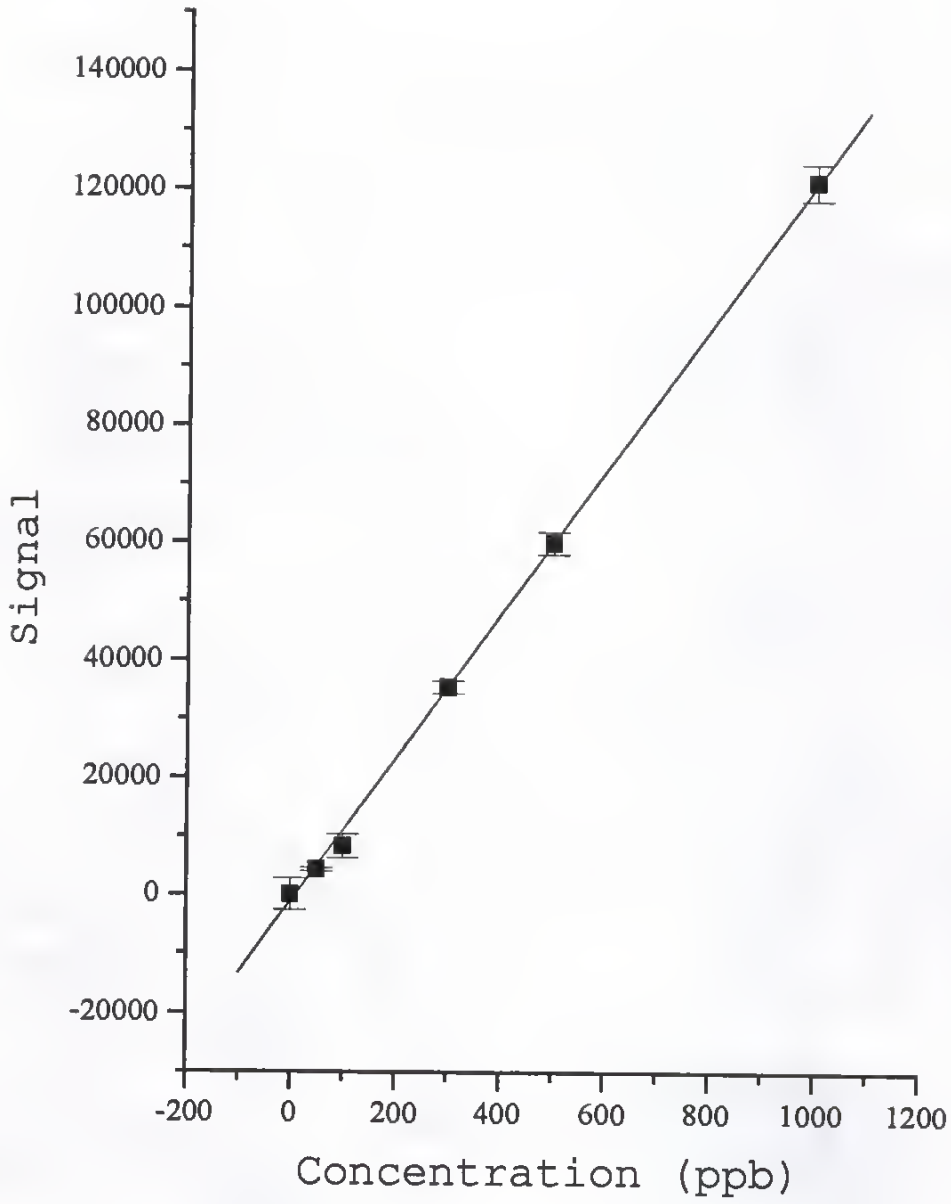


Figure 6-2. Analytical curve for aqueous lead standards on spiral electrode.

Table 6-1. Microwave plasma power settings for blood lead determination

Step	Time (s)	Power (W)	Helium Gas Flow Rate (L/min)
Drying 1	20	33	0
Drying 2	20	40	0
Drying 3	20	53	0
Ashing 1	5	30	8
Ashing 2	30	35	8
Ashing 3	30	40	8
Ashing 4	1	45	8
Atomization	9	130	8
Cleaning 1	10	250	2
Cleaning 2	10	250	8

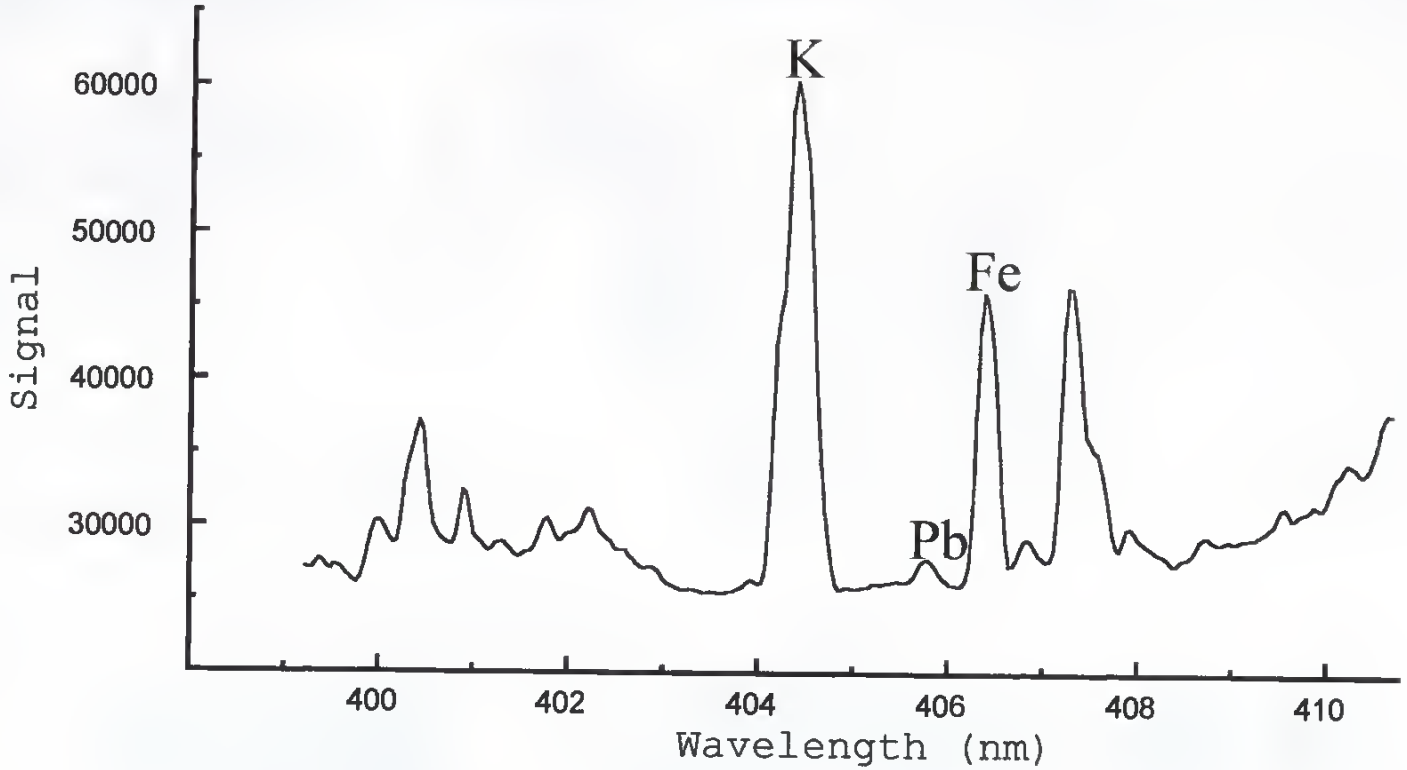


Figure 6-3. Emission spectrum (400-410 nm) for a 2 µL whole blood sample containing 411 ppb lead.

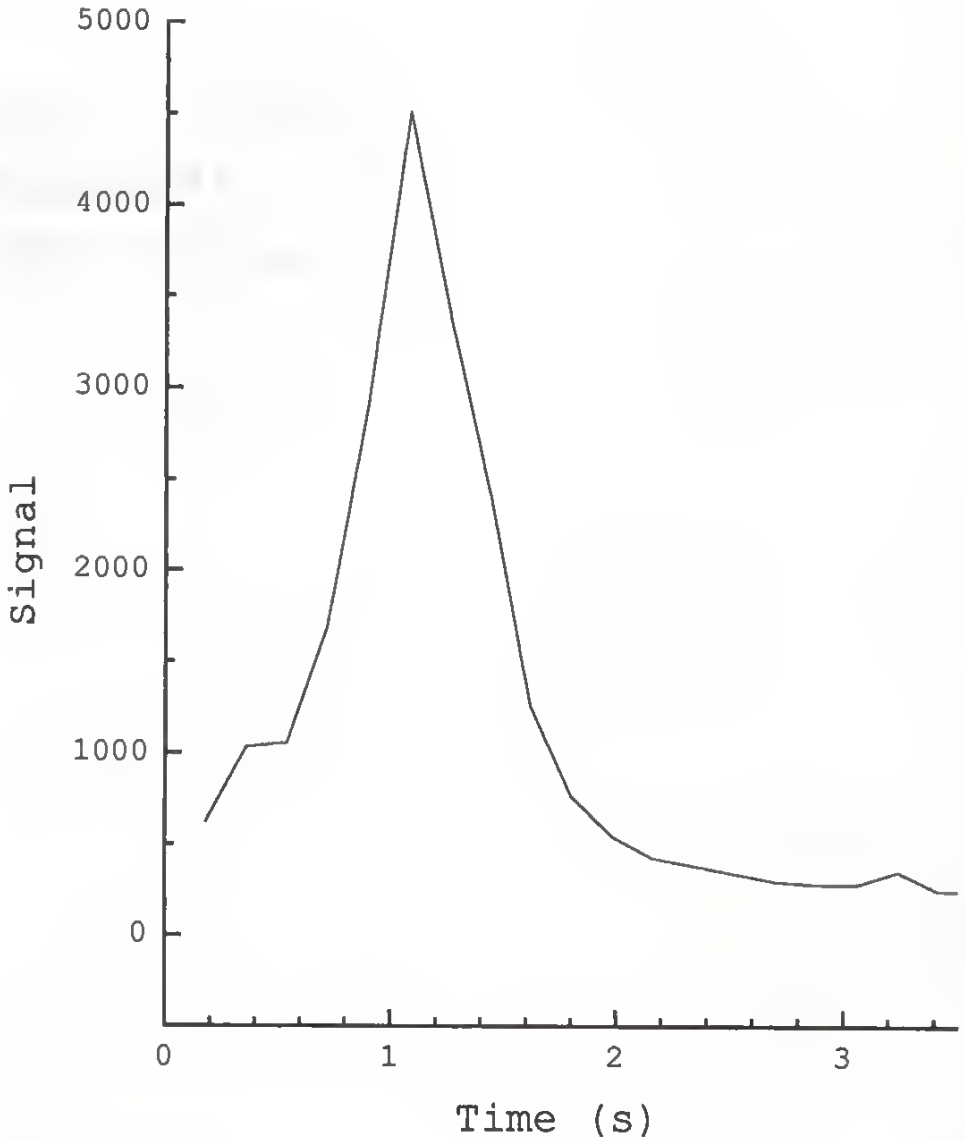


Figure 6-4. Temporal profile for lead emission signal in blood.

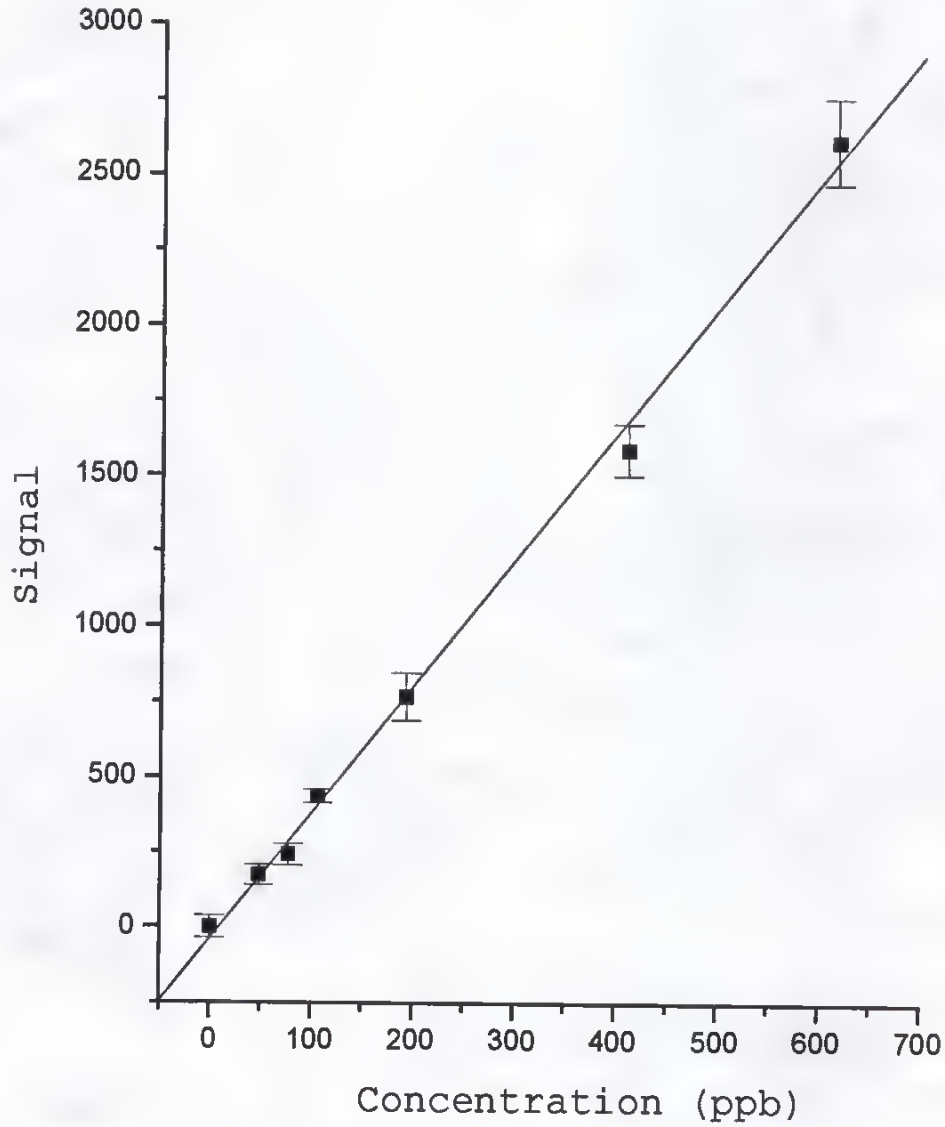


Figure 6-5. Analytical curve for bovine blood lead standards.

concentrations of ≥ 100 ppb is less than 10%. The RSD for blood lead concentrations of 20 ppb to 100 ppb is less than 20%. The analytical curve is linear with a correlation coefficient of 0.998 and a log-log slope of 0.999.

NIST Standards

The Standard Reference Materials were analyzed and compared to the analytical curve obtained from the CDC blood standards. The measured concentrations are shown next to the certified concentration values in table 6-2. The measured values agree with the certified values with 90% confidence for all the SRM's except for the lowest concentration. The accuracy at this concentration is reasonable considering that 50.1 ppb is very near the detection limit of 30 ppb.

Human Blood Standard

A human blood standard of unknown lead concentration was also analyzed using the results from the lead bovine blood standards as the analytical curve. A value of 90 ± 17 ppb was determined. The method of electrothermal vaporization-laser enhanced ionization spectrometry (ETV-LEIS) was also used to analyze the same human blood standard [143]. A lead concentration of 87 ± 3 ppb was determined by this method.

Table 6-2. Measurement of NIST lead in whole blood standard reference material.

Vial Number	Certified Concentration (ppb)	Measured Concentration (ppb)	%Bias
955a-1	50.1 ± 0.9	67 ± 20	+33.7
955a-2	135.3 ± 1.3	147 ± 30	+8.6
955a-3	306.3 ± 3.2	298 ± 24	-2.7
955a-4	544.3 ± 3.8	563 ± 20	+3.4

Blood and Aqueous Standards

A desirable characteristic of the method would be the capability of using aqueous standards for the analytical curve. The dependence of the lead signal on plasma power differed for aqueous and blood lead standards. As the atomization power for an aqueous standard was increased, the signal increased until it reached a plateau. For blood standards, however, the signal initially increased with increasing power but then reached a maximum and started to slowly decrease. At an atomization power of 130 W, the signal for both types of standards was the same as shown in figure 6-6. This would allow the use of aqueous standards for the determination of the lead levels in whole blood standards. Figure 6-7 shows an analytical curve in which both blood and aqueous standards were used. This curve shows good agreement (R=0.994) for both types of standards. The power at which the blood and aqueous signals were equal was reproducible from day to day for the same electrode, but was not reproducible for different electrodes. The spiral electrodes were made by hand, and it was difficult to make each electrode exactly the same. If they could be made commercially, the reproducibility would greatly increase, and the use of aqueous standards would be plausible.

The effect of the blood matrix on the lead signal intensity was investigated. A blood standard and an aqueous

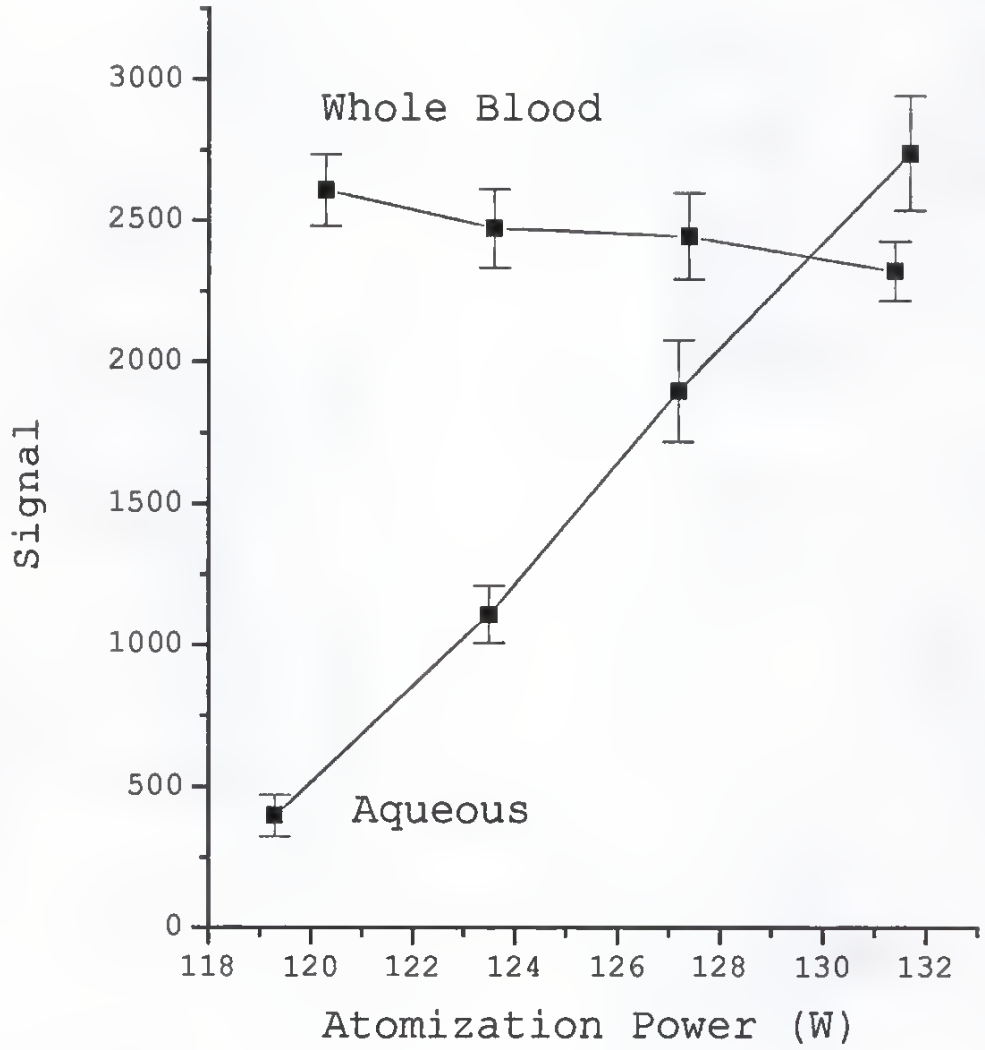


Figure 6-7. Power comparison for blood and aqueous lead standards.

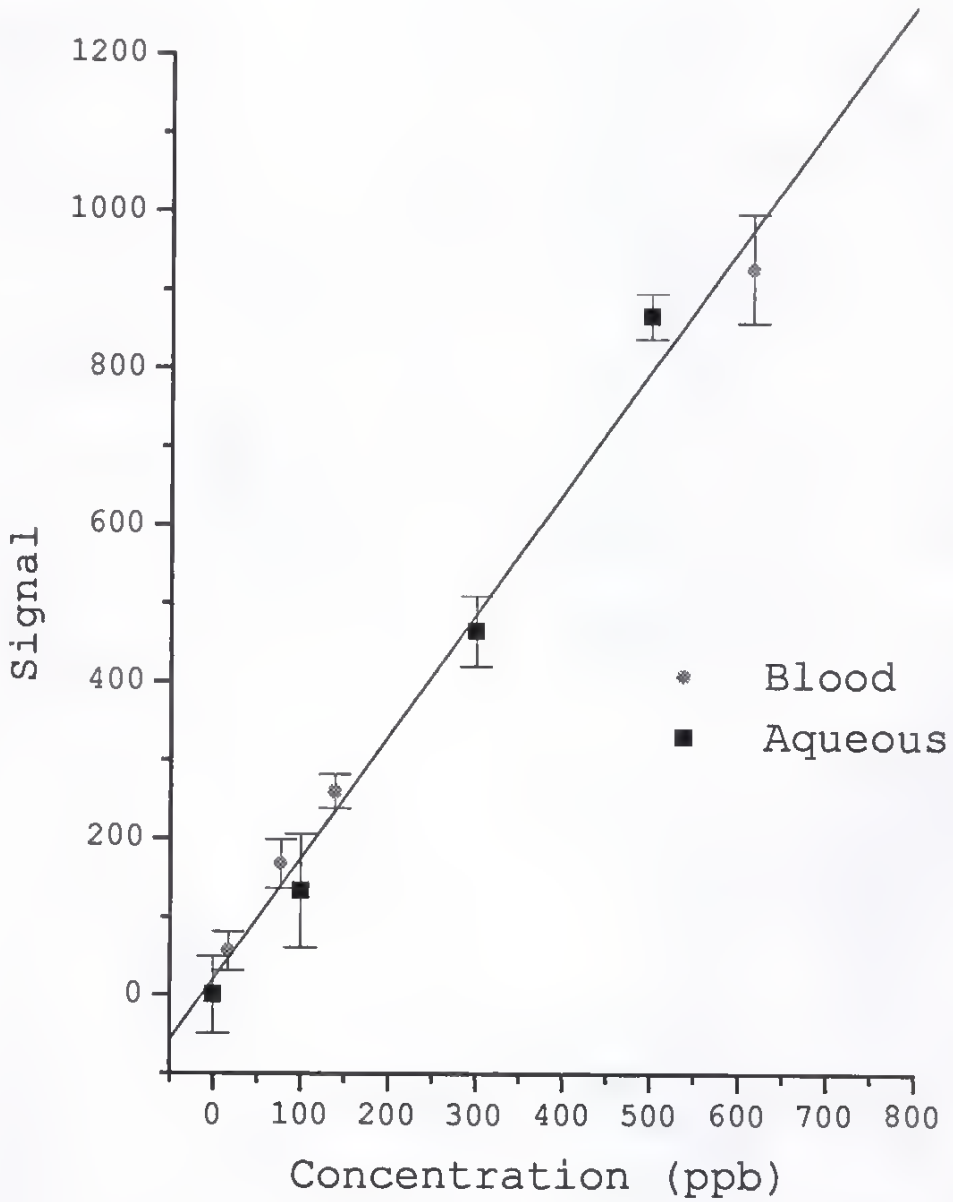
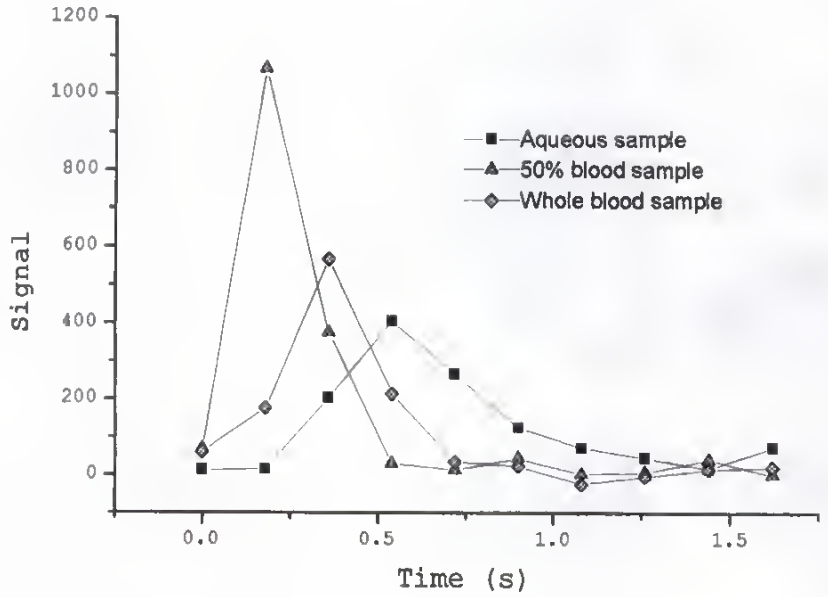


Figure 6-8. Analytical curve for blood and aqueous lead standards.

standard of the same concentration of lead were mixed in various amounts to produce blood samples that were 25, 50, and 75% whole blood. Although the sample composition was different for each standard, the concentration of lead remained the same. The intensity of lead signal for these samples and for pure blood and aqueous samples was measured at several atomization powers ranging from 131 to 250 W. The temporal profiles for the whole blood, the 50% blood, and the aqueous sample at atomization powers of 131 and 250 W are shown in figure 6-9. At all the atomization powers used, the signal for the samples containing blood appeared quicker and lasted a shorter time than the aqueous sample. This could be due to species in the blood that increase the volatilization rate of the lead present. This could explain how at the lower atomization powers, the samples containing some blood in them have greater signals than the corresponding aqueous standard. By making the lead more volatile, the processes of atomization and excitation are more efficient. As the atomization power was increased, this effect was minimized. The amount of lead signal reached a maximum because the maximum amount of lead analyte was atomized. Increasing the power did not increase the number density of lead atoms in the plasma. Also, if something in the blood was increasing the rate of volatilization of the lead, a significant portion of the

a)



b)

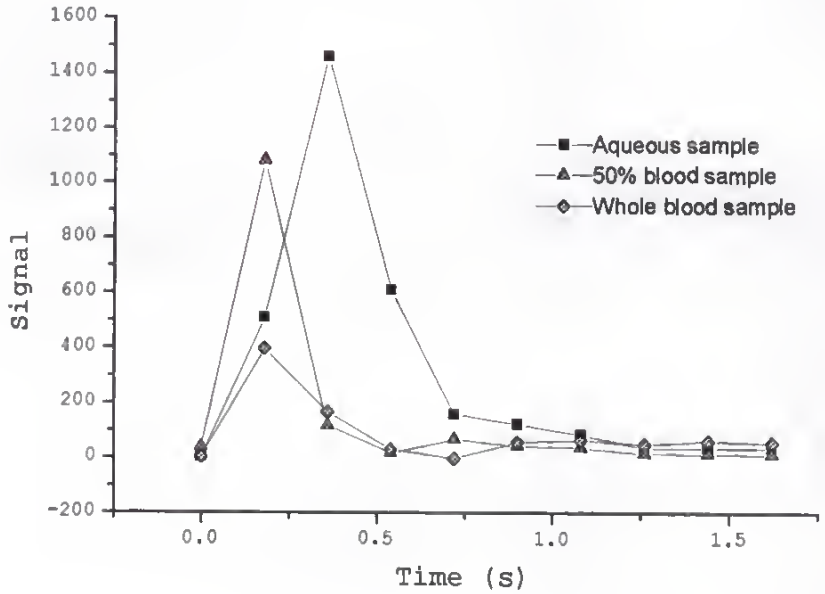


Figure 6-9. Temporal profiles for the lead signal in samples of varying blood composition at atomization powers of a) 131 W and b) 250 W.

lead analyte could be lost during the ashing step. This would explain why the signal for aqueous samples increased beyond that of the blood samples for high atomization powers.

Conclusion

The current system satisfies or approaches many of the requirements of the CDC. The accuracy and precision approach ± 10 ppb at 100 ppb with a lower detection limit approaching 20 ppb. The required blood specimen volume is only 2 μL, and the analysis time is under 4 minutes. The system can easily be automated so that operator training will be minimal and the instrument has low operating costs. The two characteristics that the instrument does not fully meet are portability and cost. The requirement of helium tanks and the size of all the instrument components limits the portability. The microwave electronics are very inexpensive because of the commercial production of the microwave oven; however, the PDA or CCD currently used as the detector makes the system expensive. This type of detector is not necessary for detecting only one element, so less expensive detection systems could be used.

CHAPTER 7
MULTIELEMENT ANALYSIS IN BLOOD

Introduction

Although the CMP-AES did not meet all the requirements for a screening method, it may still be useful as a clinical method. The method of atomic emission allows the measurement of many elements. Figure 7-1 shows an atomic emission spectrum of whole blood. There are many atomic emission lines available to analyze the various elements in blood. The CMP-AES was used to analyze the levels of sodium, potassium, magnesium, manganese, zinc and lithium in blood. The level of sodium and potassium is very high in blood, so it was necessary to determine which atomic emission lines will give linear responses in the concentration range of interest. The primary advantage of this method is that no dilution or sample pretreatment of whole blood is necessary for analysis, so it is not practical for us to dilute the blood samples in order to be able to use the strongest atomic emission lines. The initial work for each element was accomplished using aqueous standards.

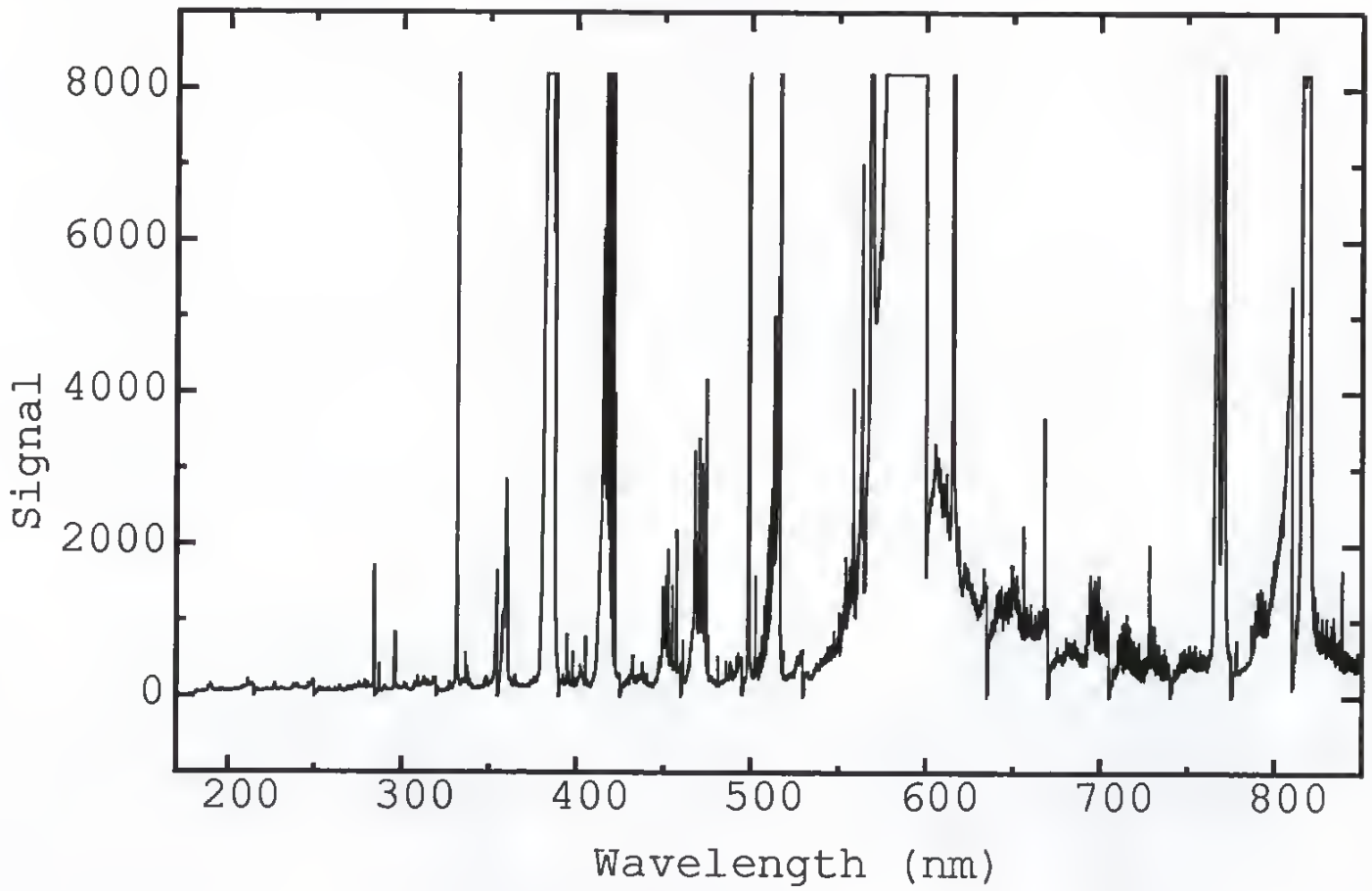


Figure 7-1. Emission spectrum of whole blood

The method of standard additions was used because currently there is no suitable blood standard for elements other than lead. Statistically, the useful concentration range of standards added to the sample should result in a final concentration of sample between 1.5 to 3 times the original concentration of analyte [7]. For standard additions analysis it is sufficient to analyze two samples, a sample without any standard added and a spiked sample. To insure linearity for the elements studied, a minimum of three samples were analyzed.

Trace Elements

Zinc

The first atomic emission line used for zinc was 636.2 nm. A limit of detection (LOD) of 140 ppb for aqueous samples at an atomization power of 170 W was determined using this wavelength. The analytical curve lost its linearity above a concentration of 2 ppm. Lower atomization powers of 130 W and 90 W were tried, but the analytical curve still was not linear at higher concentrations. Zinc was present in blood at concentrations greater than 2 ppm, and so a weaker atomic emission line had to be found. The line at 472.2 was used and was linear over the concentration range of interest. A LOD of 70 ppb was determined for

aqueous standards (figure 7-2) at an atomization power of 150 W. Figure 7-3 shows the analytical signal for zinc in blood. The LOD for zinc in blood was 2 ppm at an atomization power of 70 W. The detection limit remained approximately the same (1 ppm) for an atomization power of 165 W. The measured concentration of zinc in human blood was 8.5 ± 1.4 ppm at an atomization power of 70 W (figure 7-4) and 8.8 ± 3.2 ppm at an atomization power of 165 W.

Lithium

The atomic emission line at 670.8 nm was used for the analysis of lithium in blood. The analytical curve for aqueous standards is shown in figure 7-5. The data point from the 500 ppb lithium standard was not used for the linear fit because it is not in the linear region of the analytical curve. A LOD of 10 ppb was determined for lithium in aqueous standards at an atomization power of 70 W. When the blood standards were analyzed, a significant background appeared surrounding the lithium peak (figure 7-6a). A red glass filter was placed in front of the spectrometer and this greatly reduced the background (figure 7-6b). The measured concentration of lithium in blood was 2.6 ± .2 ppb with a detection limit of 0.6 ppb (figure 7-7).

The measured concentration of lithium in blood corresponds with reference values. However, the amount of

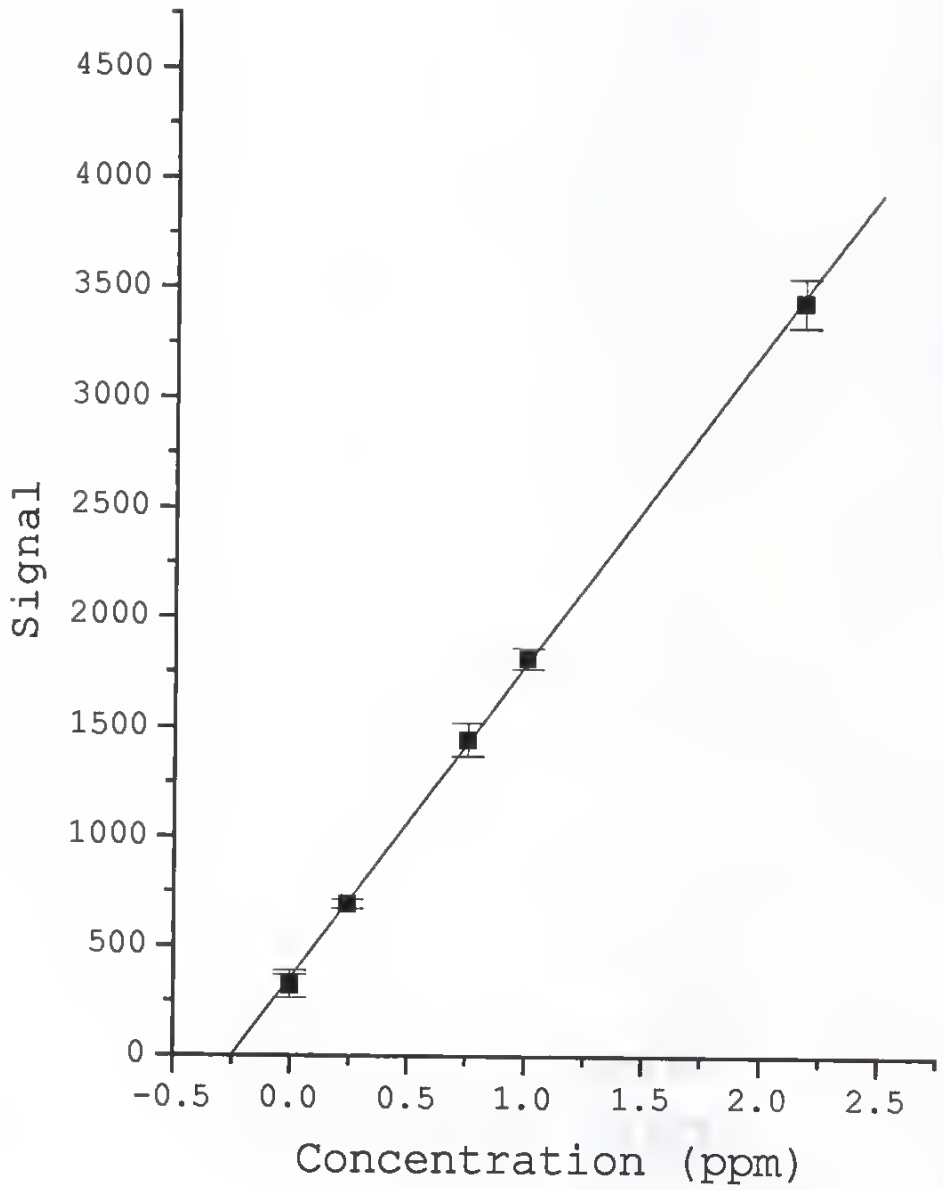


Figure 7-2. Analytical curve for aqueous zinc standards at a wavelength of 472.2 nm and an atomization power of 150 W.

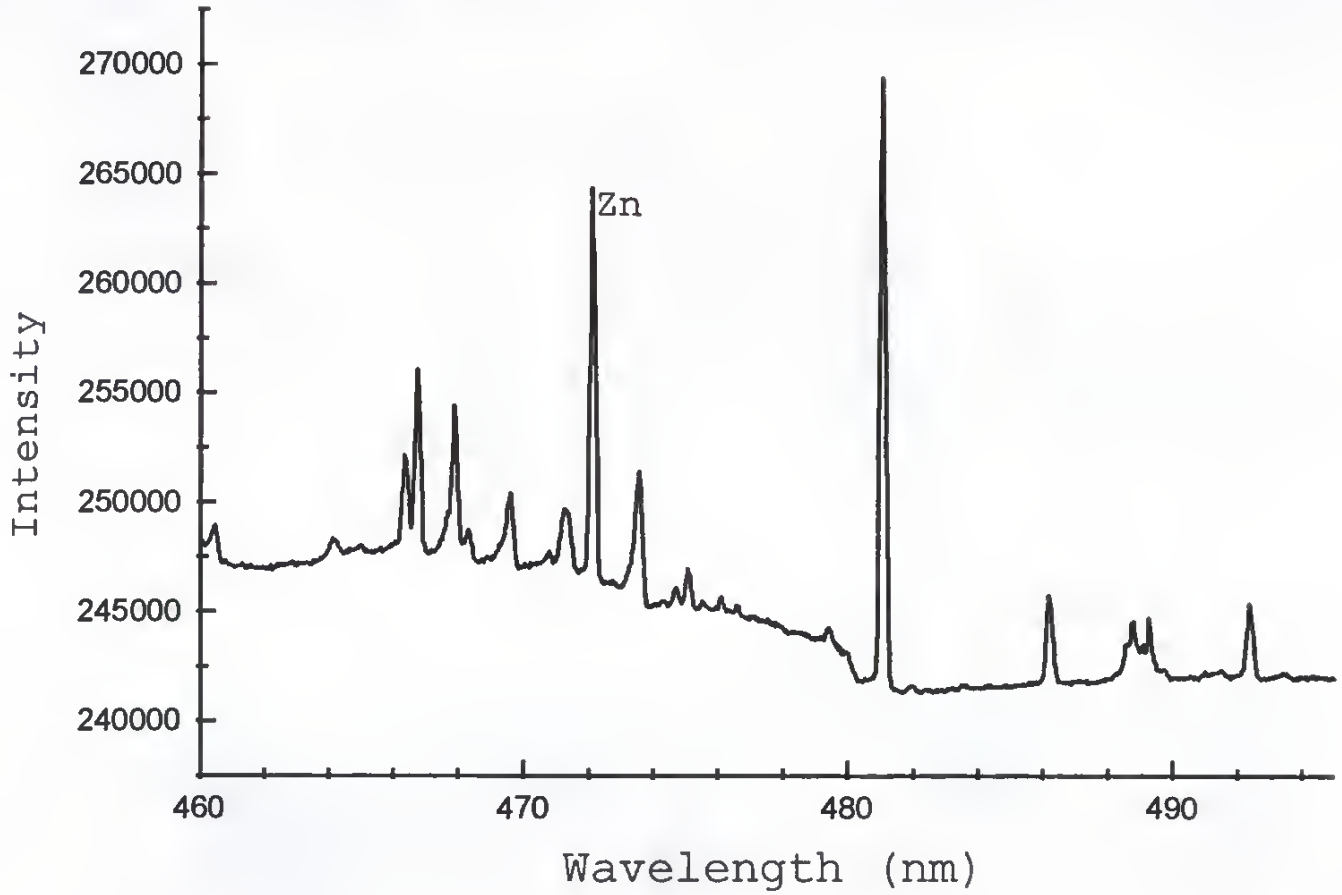


Figure 7-3. Spectrum of a 2 μL, 10 ppm, zinc blood sample at an atomization power of 70 W.

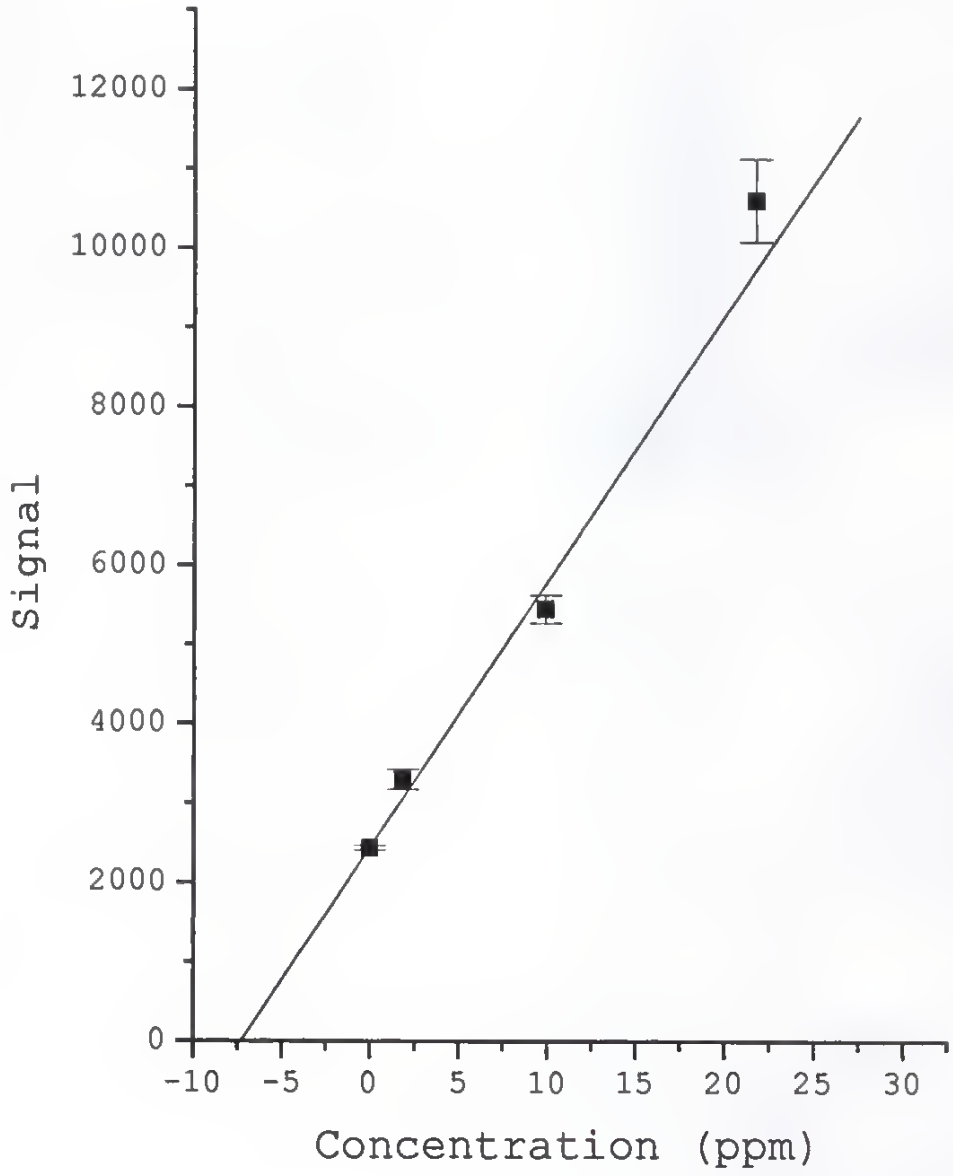


Figure 7-4. Standard additions for zinc in blood at a wavelength of 472.2 nm and an atomization power of 70 W.

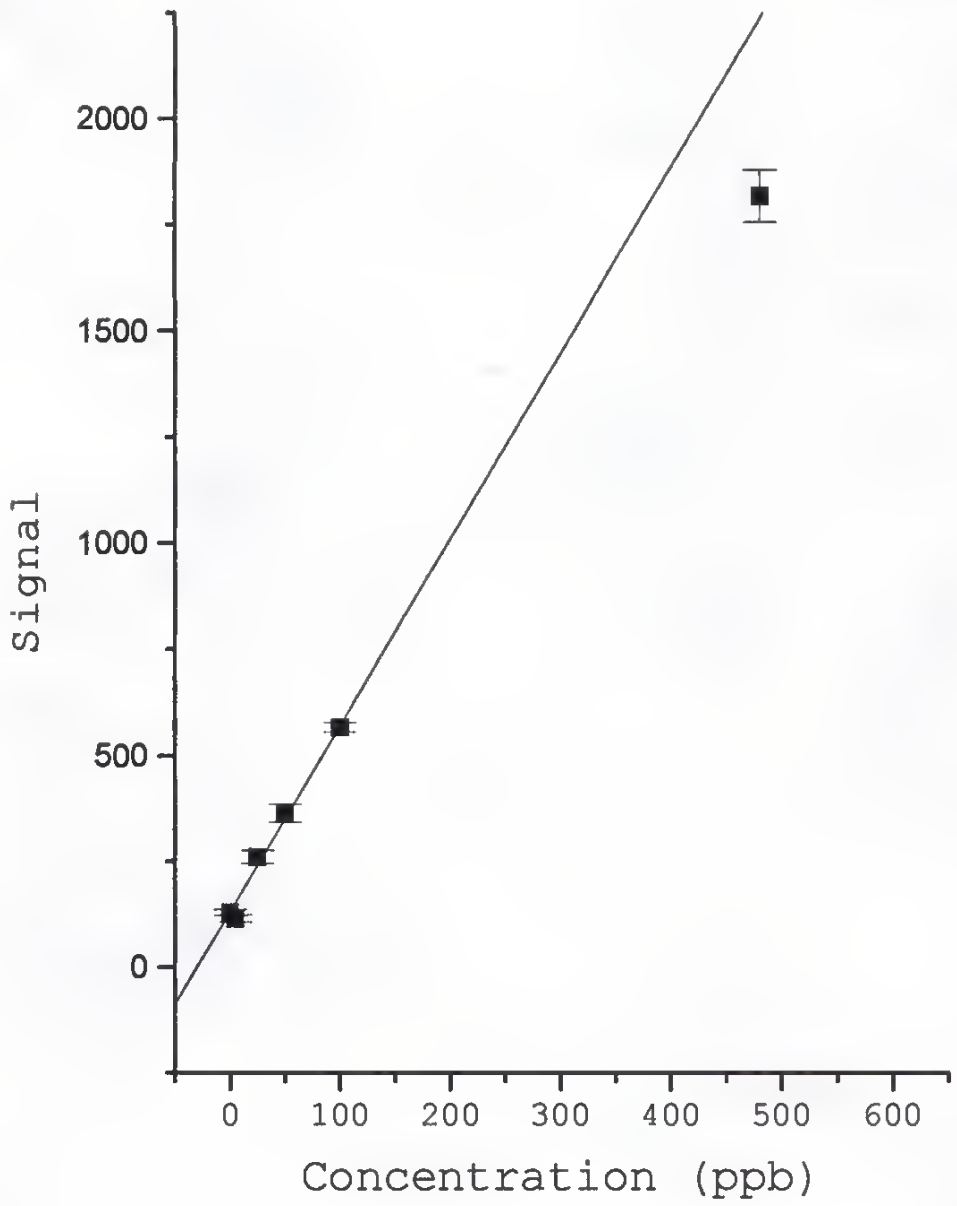
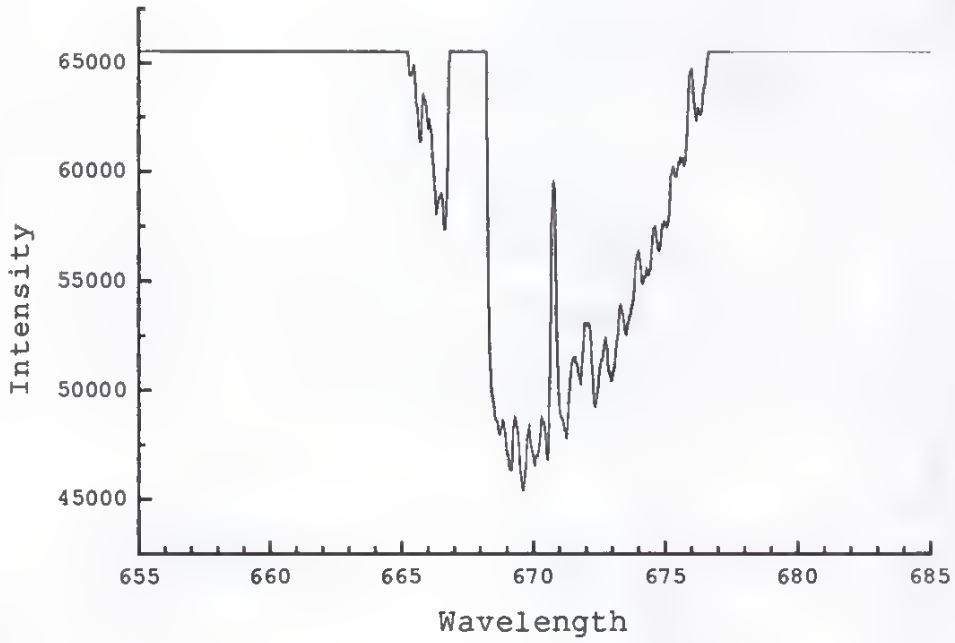
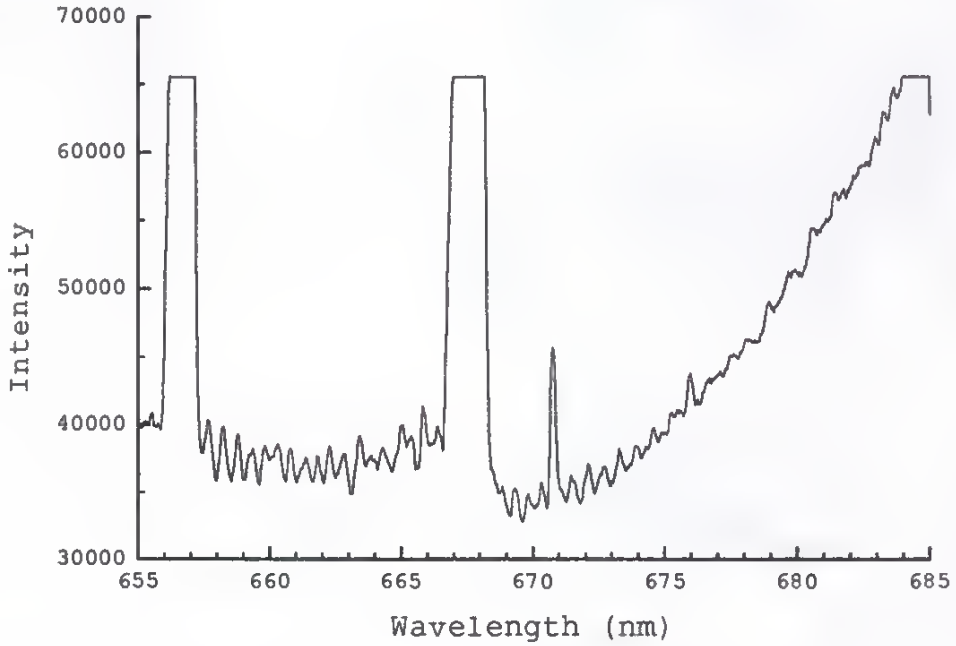


Figure 7-5. Analytical curve for aqueous lithium standards at a wavelength of 670.8 nm and an atomization power of 70 W.



a)



b)

Figure 7-6. Spectra of a 2μL, 5 ppb lithium blood sample at an atomization power of 70 W before (a) and after (b) addition of red glass filter.

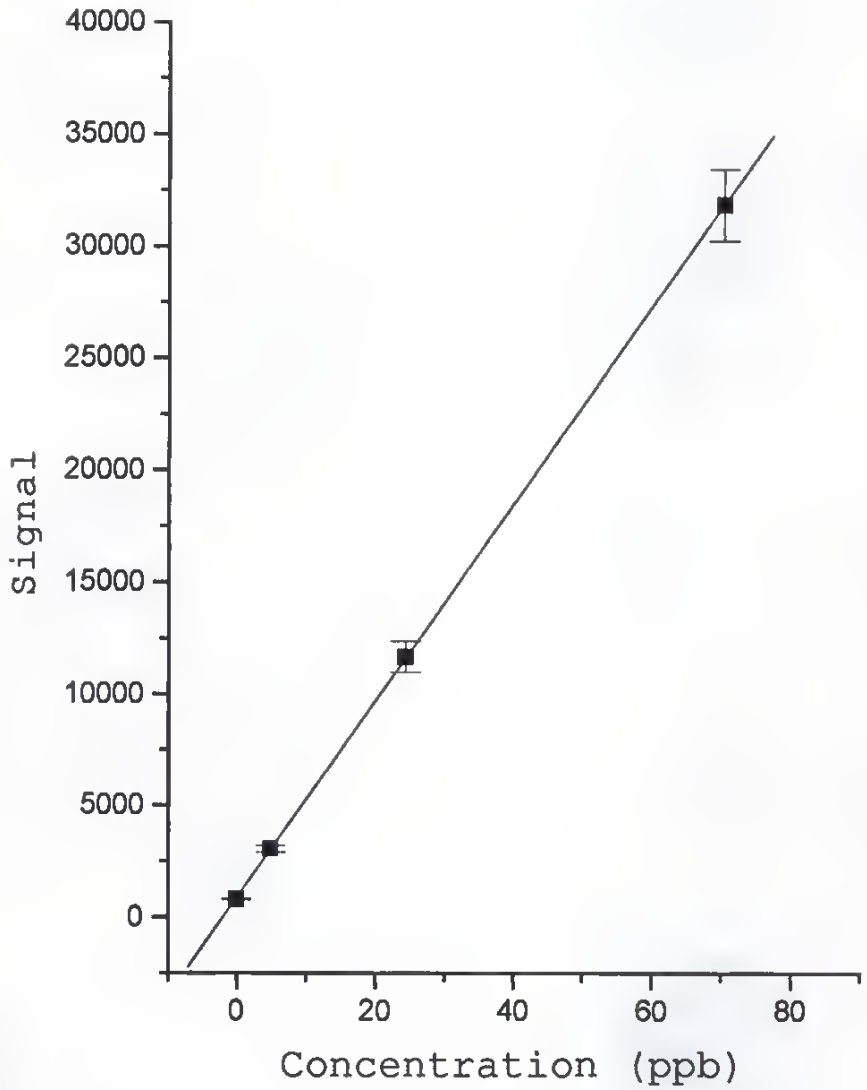


Figure 7-7. Standard additions for lithium in blood at a wavelength of 670.8 nm and an atomization power of 70 W.

lithium in the blood of patients receiving lithium treatment is usually several ppm. A weaker lithium line at 610.4 nm was used to investigate the linearity of analysis in this higher concentration range. Aqueous standards were first analyzed using an atomization power of 115 W. Figure 7-8 shows that the linear dynamic includes the region of interest with a detection limit of 10 ppb and a correlation coefficient of 0.9999. Blood samples containing higher lithium concentrations were also analyzed (figure 7-9). A detection limit of 30 ppb with a correlation coefficient of 0.998 was obtained. The relative standard deviation was better than 7% for all concentrations measured for both blood and aqueous standards.

Magnesium

The 518.4 nm atomic emission line was used for the analysis of magnesium (figure 7-10). The low power used for atomization of the other elements was not sufficient for the magnesium. An atomization power of 130 W was used. The LOD's were 5 ppm for aqueous standards and 6 ppm for blood. The measured level of Mg in the human blood sample was 28.7 ± .1 ppm (figure 7-11).

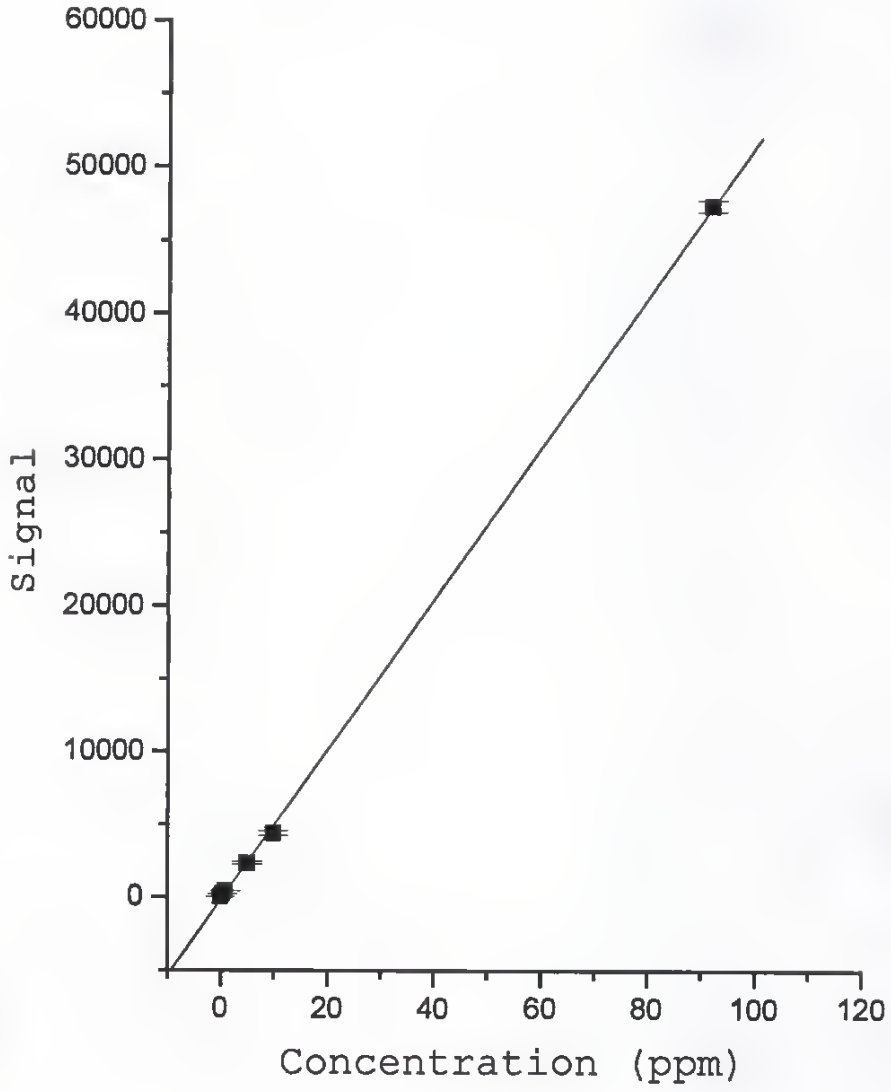


Figure 7-8. Analytical curve for aqueous lithium standards at a wavelength of 610.4 nm and an atomization power of 115 W.

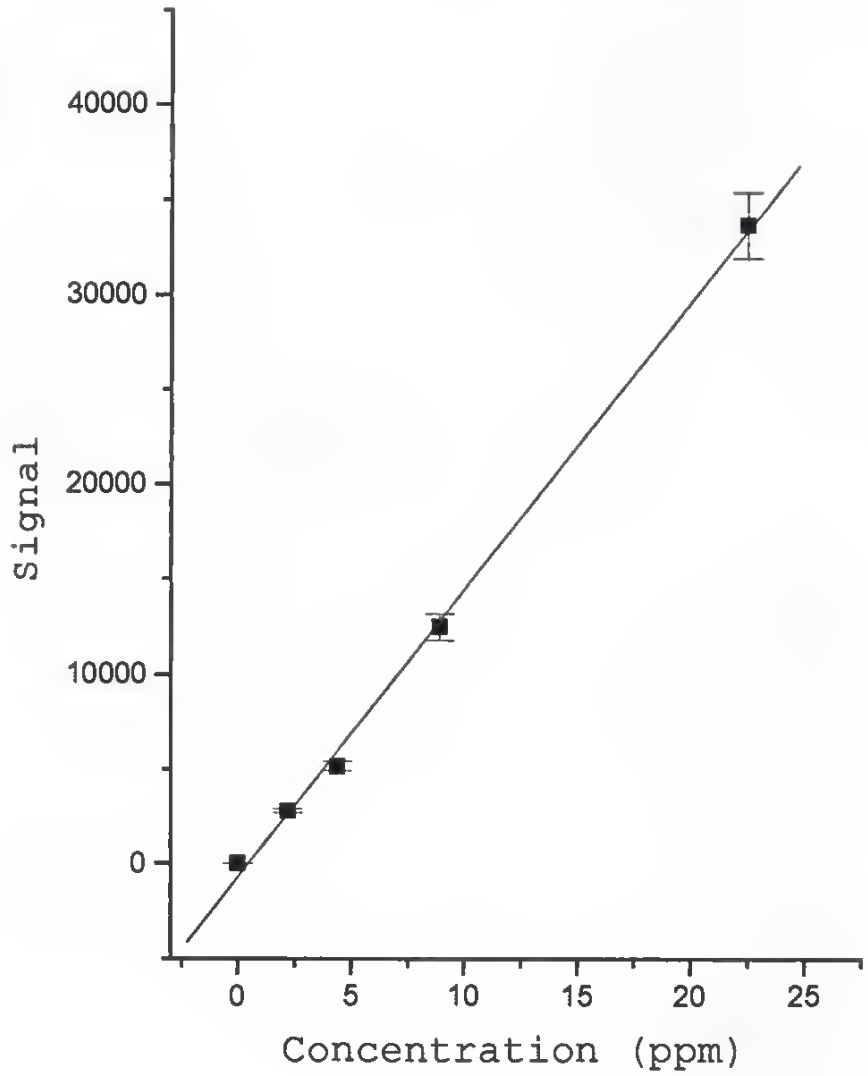


Figure 7-9. Analytical curve for blood lithium standards at a wavelength of 610.4 nm and an atomization power of 115 W.

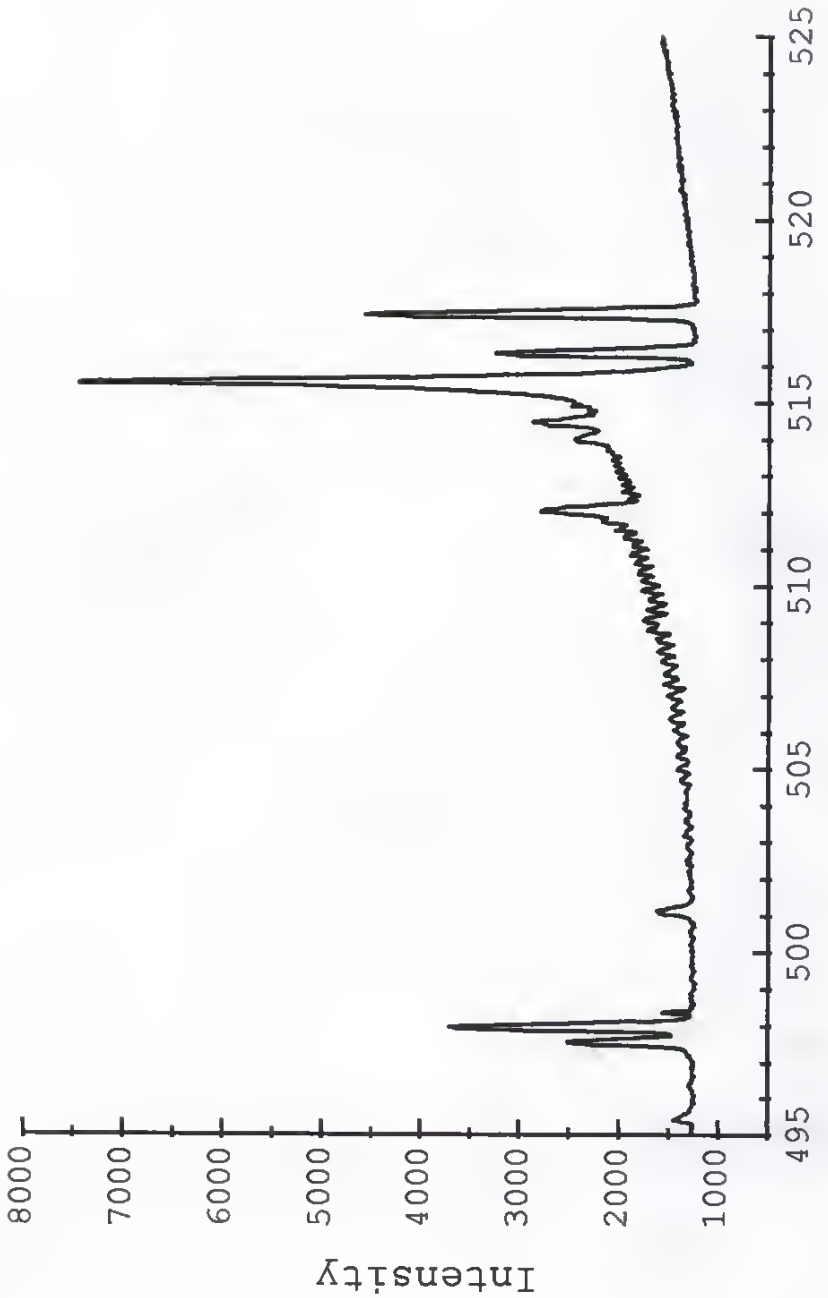


Figure 7-10. Spectrum of a 2 µL, 50 ppm, magnesium blood sample at an atomization power of 130 W.

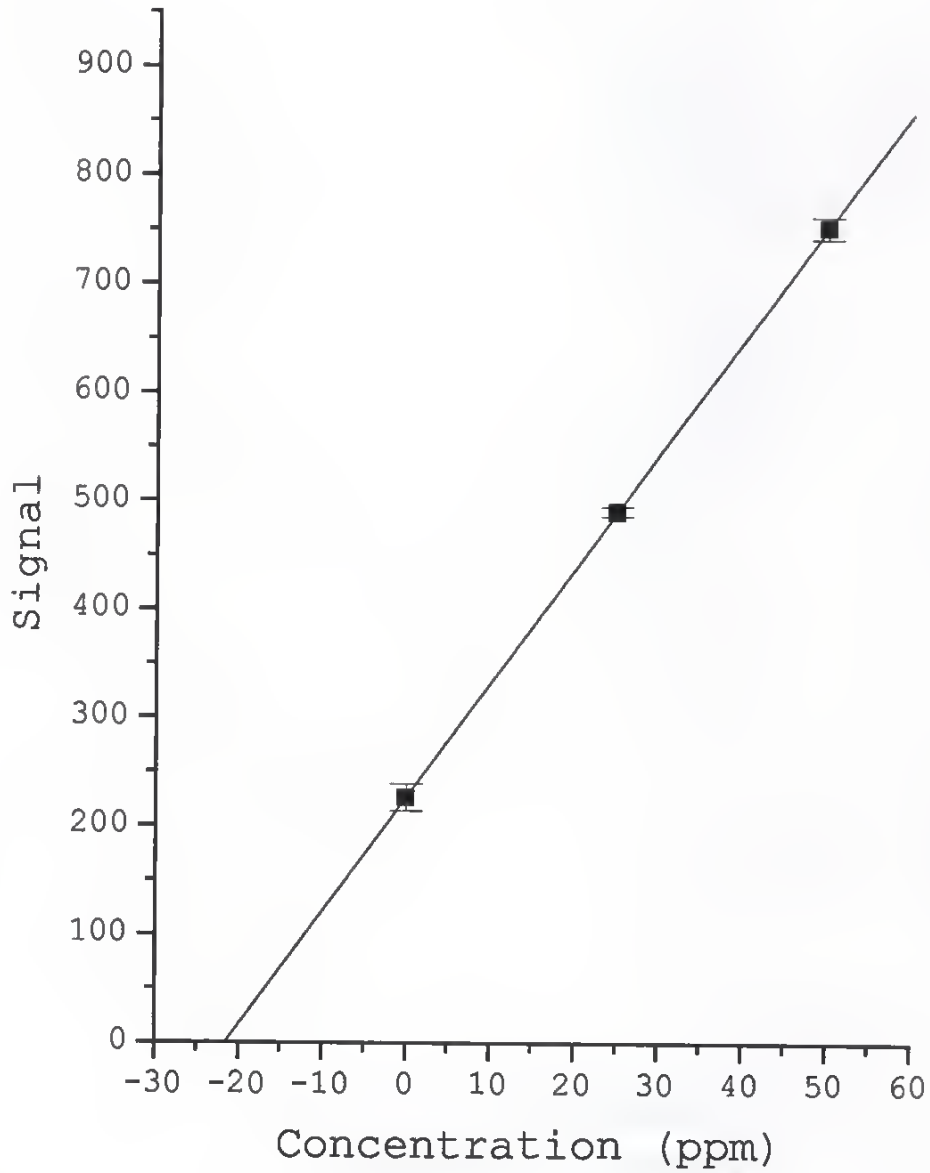


Figure 7-11. Standard additions for magnesium in blood at a wavelength of 518.4 nm and an atomization power of 130 W.

Manganese

Manganese (Mn) has three closely spaced atomic emission lines at 403.08, 403.31, and 403.45 nm. The spectrometer was not able to fully resolve these lines, so the combined area of all three peaks was used for analysis. An interfering peak appeared very close to the Mn peak and was accounted for by blank subtraction. The integration time was reduced from two seconds to one second and this reduced the amount of background without reducing the amount of Mn signal. The analysis of manganese required an atomization power of 230 W. The LOD's for Mn were 90 ppb for aqueous standards (figure 7-12) and 30 ppb for blood standards (figure 7-13). A concentration of 62 ± 8 ppb was measured in the human blood sample.

Primary Elements

Sodium

The resonance lines of sodium at 589.59 and 589.0 were investigated for use in the measurement of sodium blood levels. The analytical curve for aqueous standards (figure 7-14) was linear up to a concentration of 1 ppm. The correlation coefficient using the results for sodium concentrations up to 1 ppm was 0.999 with a log-log slope of 1.01. The detection limit was 10 ppb for an atomization

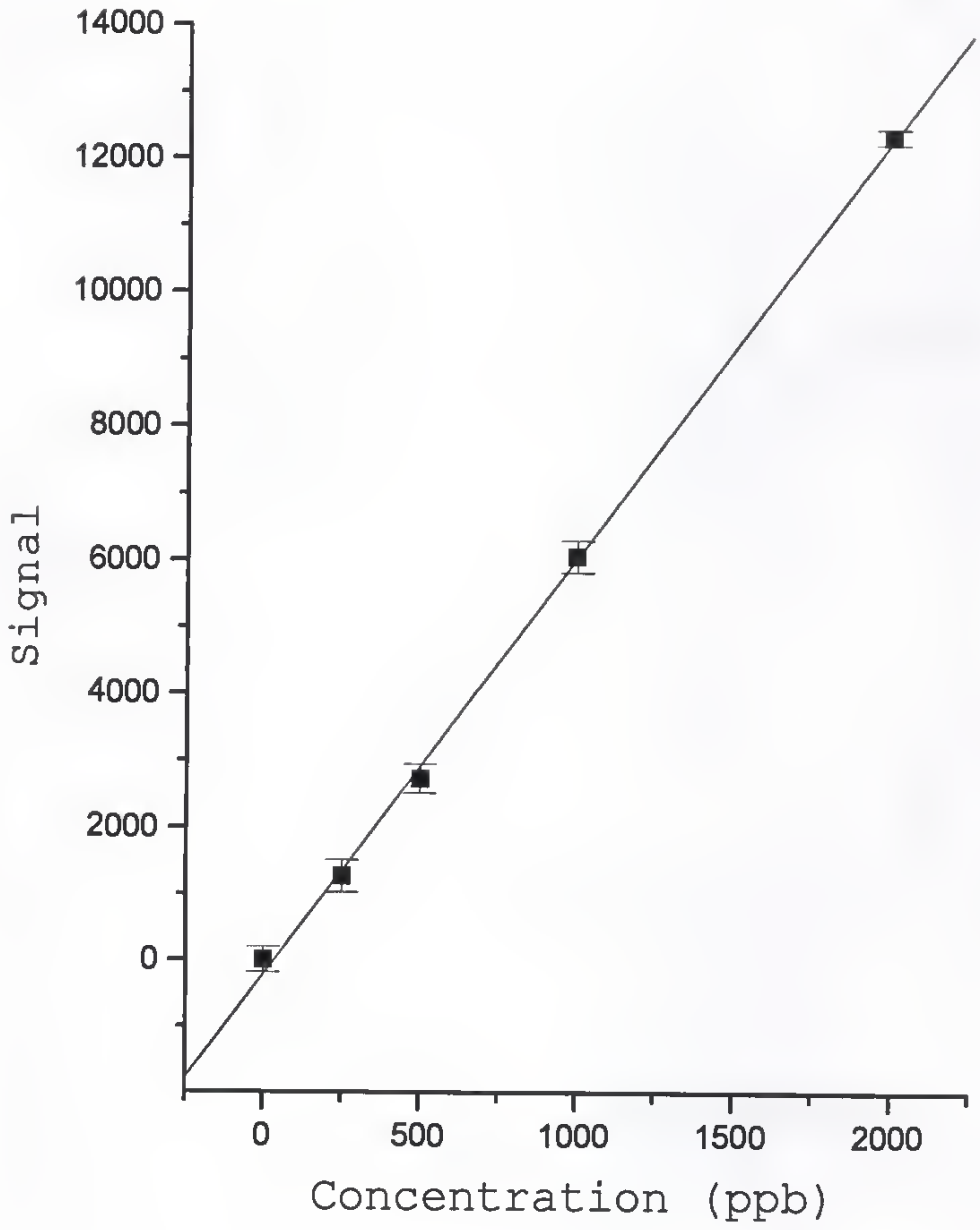


Figure 7-12. Aqueous analytical curve for manganese at a wavelength of 403 nm and an atomization power of 230 W.

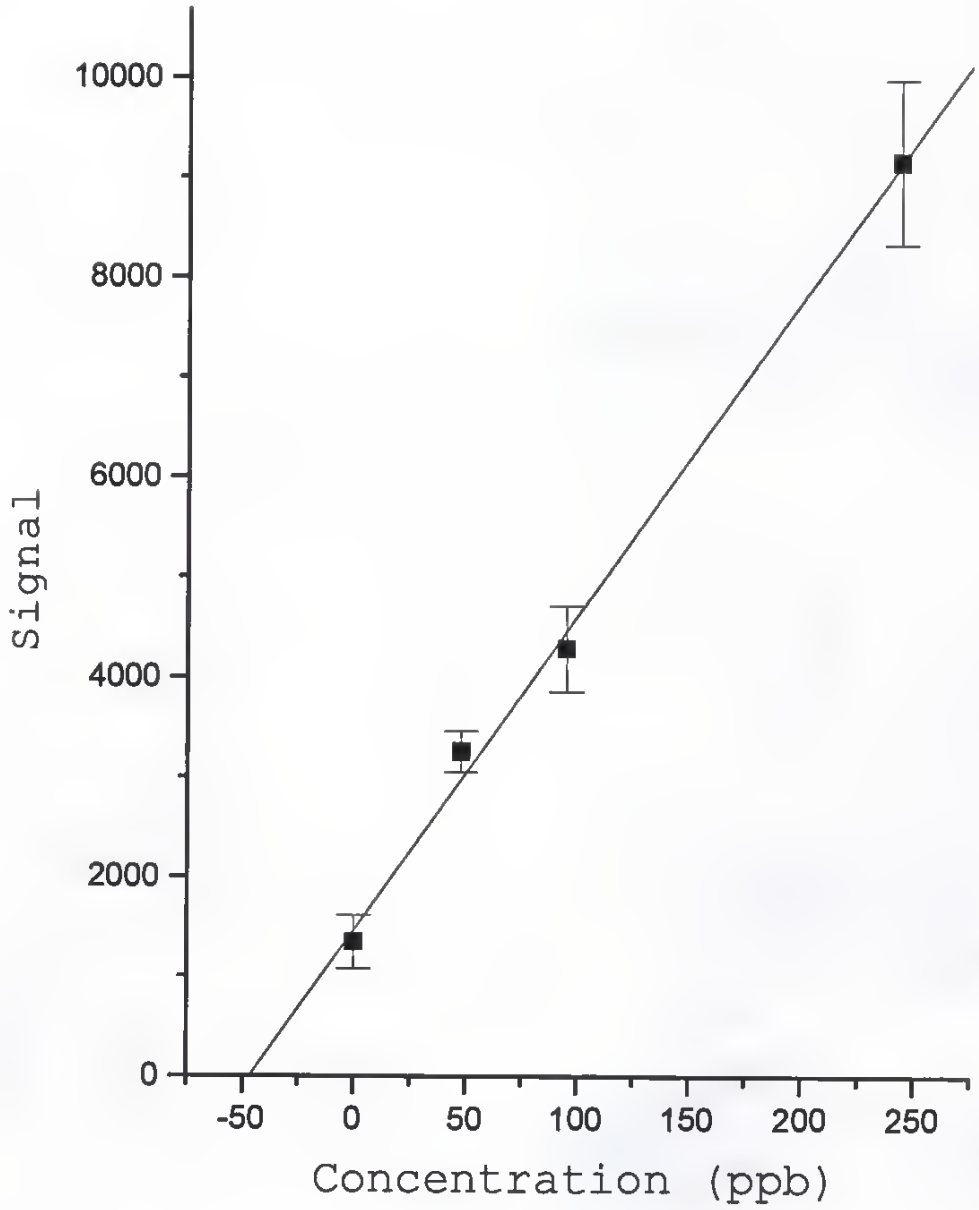


Figure 7-13. Standard additions for manganese in blood at a wavelength of 403 nm and an atomization power of 230 W.

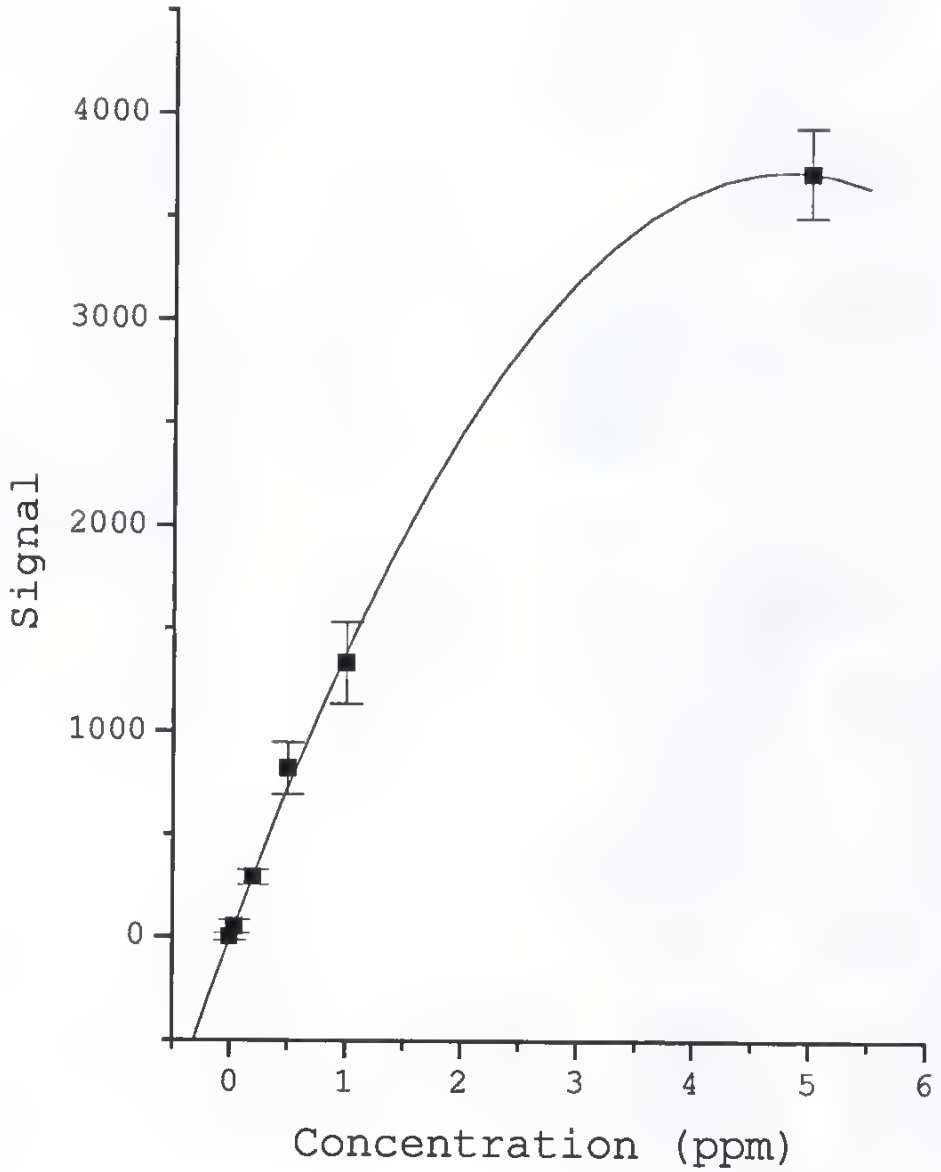


Figure 7-14. Aqueous analytical curve for sodium at a wavelength of 589 nm and an atomization power of 70 W.

power of 70 W. The concentration of sodium in blood is well above 1 ppm so other atomic lines were investigated. Sodium lines at both 568.8 and 819.5 nm were tried, but theanalytical curve for these lines was not linear at higher concentrations. The atomic emission at 498.3 nm gave linear results over the concentration range of interest so it was used for blood analysis. The sodium peaks in blood are shown in figure 7-15. Neutral density filters had to be used because the emission intensity from the sodium was too high for the detector. An LOD of 60 ppm was determined using the 498.3 nm line and atomizing at 110 mA. The concentration of sodium measured in the human blood sample was 1240 ± 60 ppm.

Potassium

The level of potassium (K) in blood was measured using the atomic emission lines at 766.49 and 769.90. Neutral density filters were used to reduce the intensity so that the detector was able to measure the potassium emission. LOD's of 30 ppb for an atomization power of 150 W and 140 ppb for an atomization power of 70 W were determined for aqueous standards. The LOD for potassium in blood was 250 ppm for an atomization power of 70 W. The measured concentration of potassium in the human blood sample was 1660 ± 120 ppm (figure 7-17).

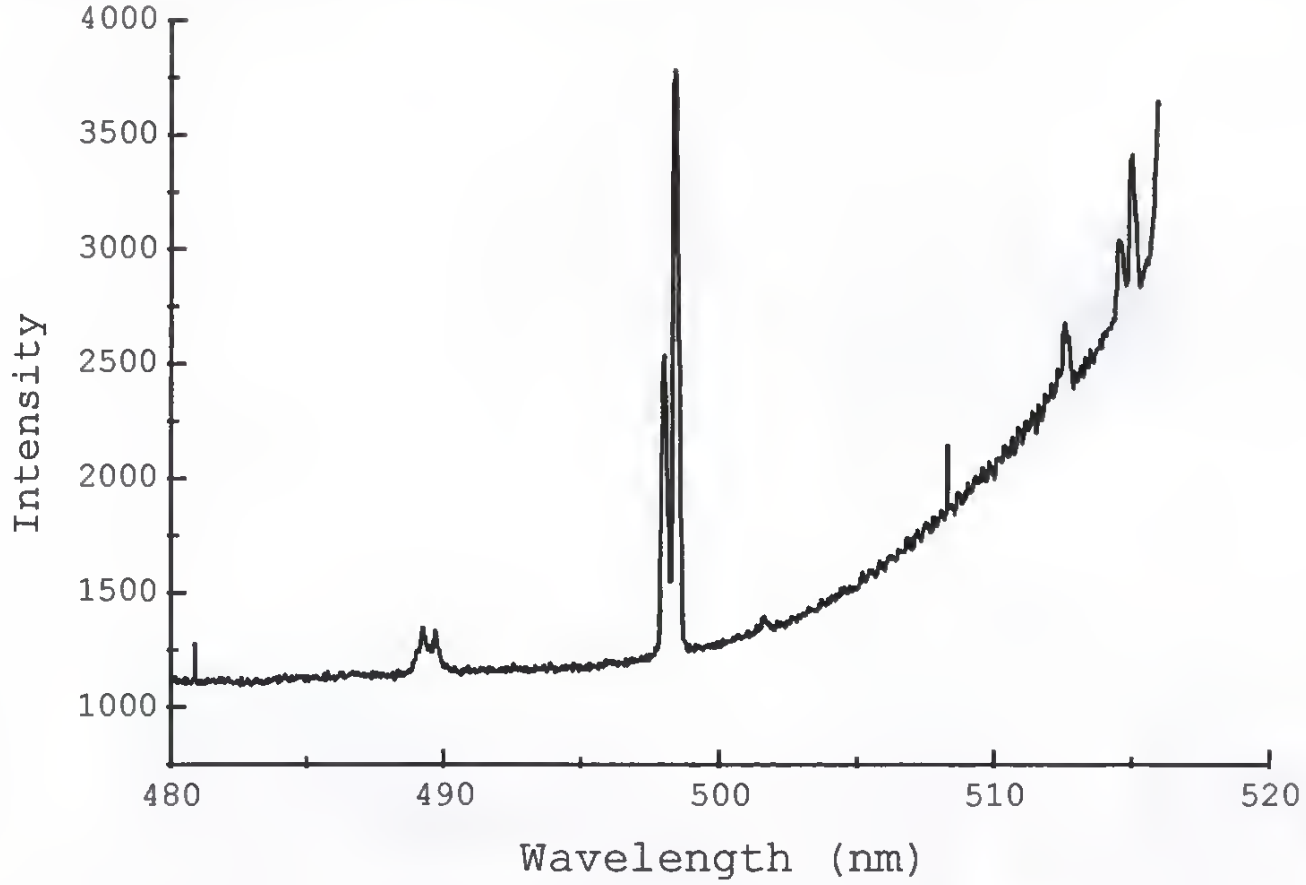


Figure 7-15. Spectrum of a 2 μL, 1900 ppm, sodium blood sample at an atomization power of 110 W.

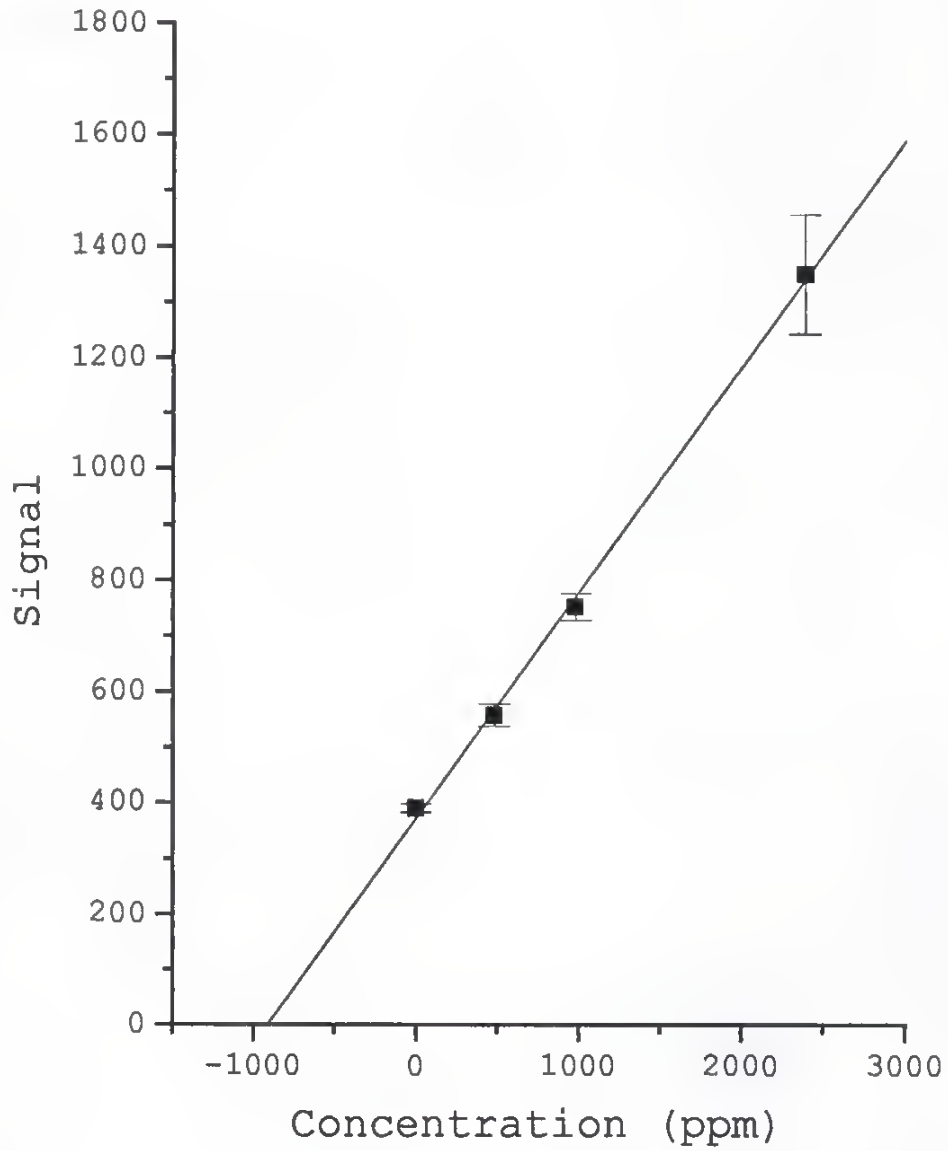


Figure 7-16. Standard additions for sodium in blood at a wavelength of 498 nm and an atomization power of 110 W.

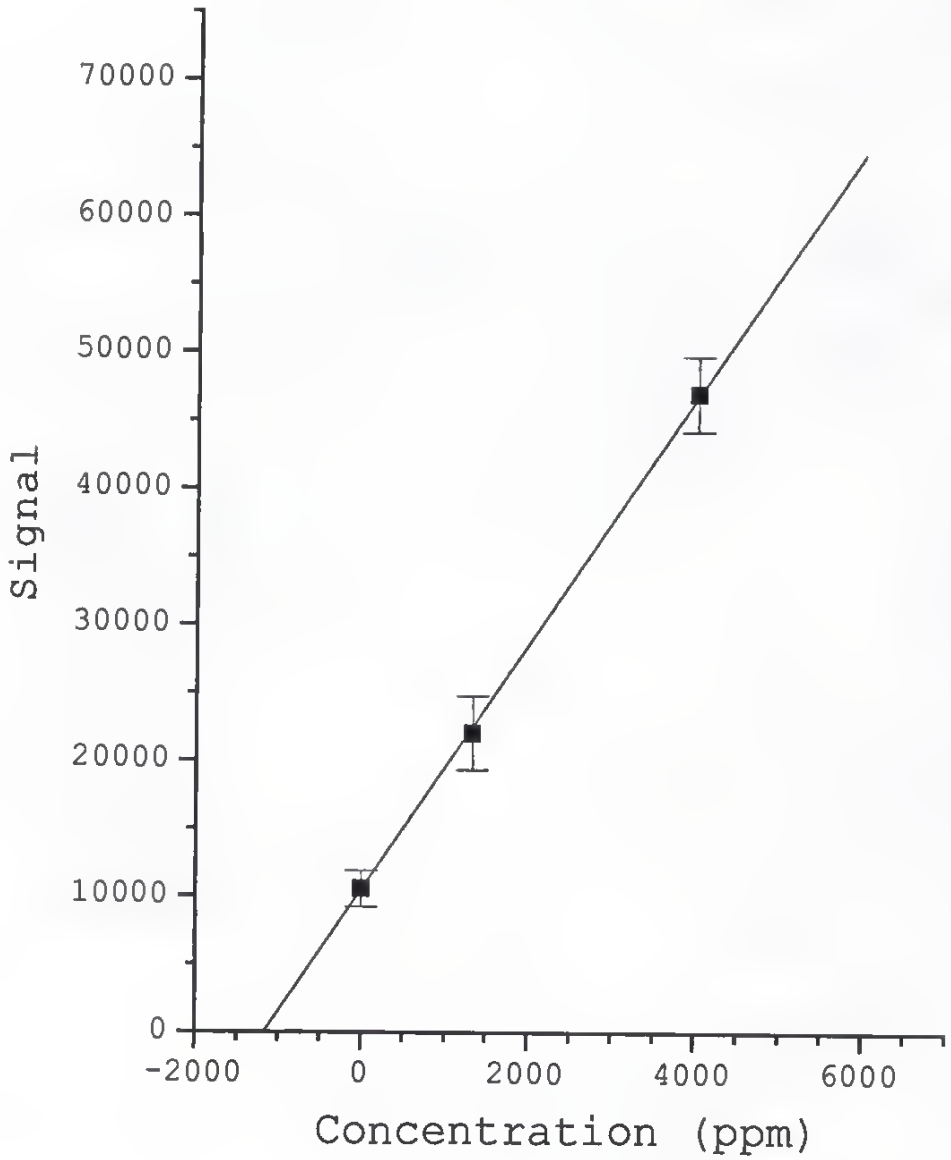


Figure 7-17. Standard additions for potassium in blood at wavelengths of 766.5 and 769.9 nm and an atomization power of 70 W.

Comparison to Literature Values

The values obtained for the different elements using the CCMP-AES were compared to literature values. The comparison is shown in table 7-1. All the values determined fall in the reference range except for sodium and lithium, which are both lower than the reference range. The level of lithium is not much lower than the reference range, and the level of sodium is of the same order of magnitude as the reference range. The source used [144] for the reference range did not claim that the values were normal, it only pooled values obtained by various researchers for elemental analysis in blood. Therefore, the elemental levels below the lowest concentration of the reference range does not indicate a deficiency, nor does a value above the highest concentration indicate an excess. Unless the determined value is significantly outside the reference range, it could still be within the range for good health. Reference ranges can be very dependent on the population studied, the techniques used for sample collection, and the analytical method used [33, 145].

Table 7-1. Comparison of measured elemental concentrations to literature values.

Element	Concentration range in blood (ppm) [144]	Measured concentration (ppm)
Sodium	1710 - 2050	1240 ± 60
Potassium	1450 - 1920	1660 ± 120
Magnesium	27.1 - 45.5	28.7 ± .1
Lead	0.088 - 0.40	0.090 ± .017
Zinc	4.8 - 9.3	8.5 ± 1.4
Manganese	0.0016 - 0.075	0.062 ± .008
Lithium	0.003 - 0.038	0.0026 ± .0002

Conclusion

The CMP-AES was successfully able to measure the levels of sodium, potassium, magnesium, lithium, zinc, and manganese in blood. The precision for most concentrations was better than 10% and the linearity was excellent over the concentration range of interest. The detection limits for all the elements except for manganese were below the range of concentrations found in human blood.

CHAPTER 8
CONCLUSIONS AND FUTURE WORK

Conclusions

CMP-AES as a Lead in Blood Screening Method

The CMP-AES currently satisfies most of the Centers for Disease Control's requirements for a screening method for lead in blood. It is capable of detecting blood lead levels accurately below the current level of concern. The limit of detection for lead in blood by the CMP-AES is near that obtained by ICP-AES [146]. The CMP-AES has the advantage over other methods that it can analyze whole blood directly, without any sample pretreatment or dilution. Limitations of its use include the high initial cost for the instrumentation, difficulties in transporting it (i.e. to schools), and the requirement of a compressed gas source.

CMP-AES as a Multielement Clinical Technique

The CMP-AES is a technique with sensitivity to analyze trace elements and the linear dynamic range to measure primary elements. The instrumentation is inexpensive compared to GFAAS and ICP-MS, but it is expensive compared

to the electrochemical methods. CMP-AES has the advantage over electrochemical methods that reagents are not needed. The CMP-AES has several disadvantages for blood analysis. Interfering emission lines from the electrode material can make the analysis of some elements difficult. When the electrode wears down, it must be replaced and then it is necessary to recalibrate the system because the electrodes are not reproducible. Also, the electrodes are difficult to make because the tungsten is somewhat brittle. Currently, the only way to analyze blood samples is by the method of standard additions. This adds to the analysis time and is a potential source of contamination. If aqueous standards could be used for calibration, the method would be clinically useful.

Future Work

The method has proven to be capable of analyzing the elements studied, but further research is necessary to develop the method into something that could be commercially made for clinical analysis. The method could also be used in a number of other research projects.

Analysis of Other Health Related Elements

Other essential trace elements that could be studied include iron, calcium, chromium, cobalt, copper, molybdenum, and selenium [36].

Iron (Fe) deficiency is one of the most widespread nutritional deficiencies world wide [36]. Researchers at the National Center for Health Statistics (Hyattsville, Md.) estimate that 700,000 infants and 7.8 million women are iron deficient [56, 147]. It is estimated that over one third of these cases have iron deficiency anemia. If occurring during infancy and early childhood, iron deficiency anemia (IDA) can impair psychomotor development [148]. IDA also causes a lack of energy, low birth weight, prematurity, impaired immune response, and abnormalities of the skin, nails, and mucous membrane [148]. Any time a person loses a significant amount of blood, he or she must make up for the blood lost and will require iron. Vegetarianism can lead to iron deficiency because meat and fish are better sources of iron than vegetables and help in the absorption of iron from other foods [53].

Iron metabolism is intimately tied up with the metabolism of other trace elements. Its main role is in the structure of red blood cells, which provide the oxygen supply to the rest of the body's cells. Approximately two thirds of the body's iron is tied up in hemoglobin in the

red blood cells. Iron is also found in transport proteins such as transferrin and ferritin.

Abnormally high levels of iron can cause damage to several organs including the liver, heart, pancreas, and pituitary gland. High levels may also cause ischemic heart disease and cancer [148].

Calcium is important in the prevention of osteoporosis and hypertension. Calcium is also very important for the growth of children, and expectant mothers need to have a sufficient supply for their unborn baby.

Chromium is essential for normal carbohydrate metabolism [149-151]. Chromium is also a potentiator for insulin action. A deficiency of chromium could lead to insulin resistance [47].

Copper is involved in the immune and anti-oxidative defense mechanisms and in tissue repair. Irregular levels of copper have also been associated with several diseases [47].

Elements that exhibit toxicity at high levels include aluminum and cadmium. Aluminum can be toxic if the levels are too high. Aluminum can permeate food cooked in aluminum cook ware and it is also found in table salt, antacids, and deodorants [36]. An aluminum overload contributes to anemia and can cause nervous system toxicity. Aluminum has also been linked to Alzheimer's disease [47, 152]. Monitoring

the levels of aluminum in the serum and urine of patients with renal disease is an efficient way to monitor their pathological status [153].

Cadmium toxicity is a particular risk to smokers because cigarettes yield cadmium in the smoker's lungs [154]. Cadmium displaces zinc from important enzymes making them inactive [36].

Using Aqueous Standards for Blood Analysis

The use of blood samples as standards is not very practical. Although the existence of lead in bovine blood standards from the CDC's BLLRS makes it convenient to use these standards for lead, the availability is limited to research and quality control purposes. Also, there is not such a program for other elements in blood. The standard additions method works for elemental analysis, but the sample preparation is time consuming and the addition of standards can be a source of contamination. Also, it is difficult to do simultaneous multielement analysis using standard additions.

The work done comparing aqueous and blood lead standards is promising. At the determined atomization power, good agreement was obtained for the two types of standards indicating that the method could be used for blood lead screening using aqueous standards. It is possible that

a similar relationship for atomization power could be found for other elements.

Another possibility for standards is trying synthetically prepared standards that approximate the matrix of blood. However, there are no such standards currently available.

Commercially Made Filaments

The lack of reproducibility between filaments makes finding a commercially made filament worth investigating. Tungsten filaments of various shapes and sizes are used in light bulbs. These filaments can be investigated to see if they will work with the CMP-AES system. It may be necessary to design a support made out of tungsten or titanium rod as part of the electrode. The support electrode would be immersed in the waveguide and the filament would be attached to the top of the electrode. Figure 8-1 shows a possible design for using a commercial filament. The filament is made out of tungsten and has a rectangular shape with a hollow center. The two ends of the filament could be bent at a ninety degree angle and inserted into two holes in the top of a titanium support electrode. The rectangular portion of the tungsten filament would provide a platform for the placement of the sample. The microwave energy would

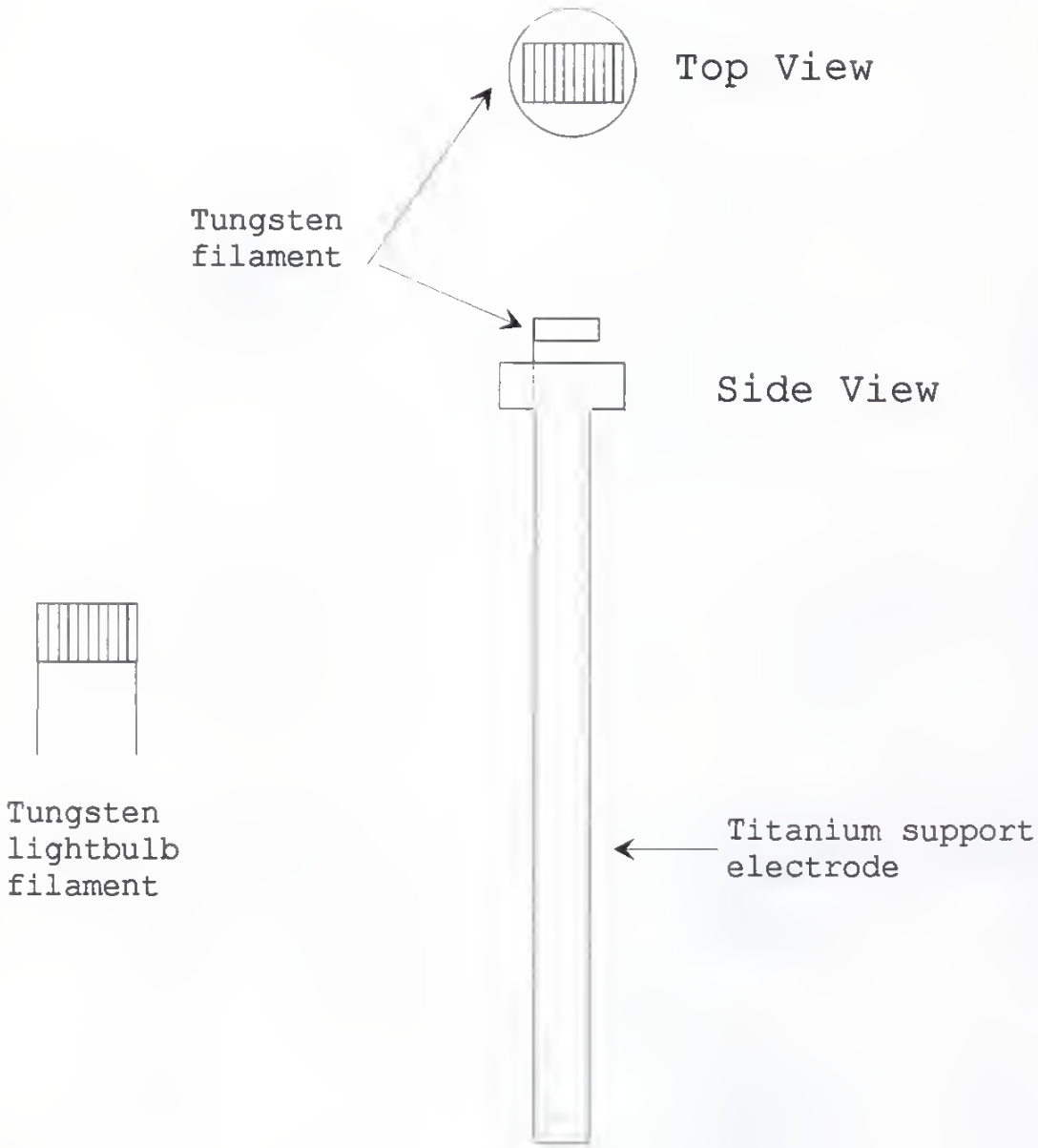


Figure 8-1. Proposed method of using a commercial tungsten lightbulb filament as the method of sample introduction.

be coupled to the filament through the support. The plasma would form either at the top of the filament or at the top of the support electrode, engulfing the filament, providing efficient atomization and excitation. If successful it could be possible to use the filaments interchangeably. The blood could then even be collected on the filament and dried and ashed using a furnace, and then stored prior to analysis. This would greatly increase the throughput of the method.

Simultaneous Multielement Analysis

An echelle spectrometer coupled with a two dimensional charge transfer device makes it possible to collect a spectral range of 500 nm. This would allow simultaneous multielement analysis using a CMP-AES. Pless *et al.* used such a system to do multielement analysis in solid samples [130]. For multielement analysis in blood samples, a compromise would be needed for the conditions of each element so that all elements could be measured simultaneously. If such a compromise could be found without a significant sacrifice of sensitivity, precision, and accuracy, then a direct elemental analysis could be performed on a single microliter blood sample. Simultaneous multielement analysis of blood would be very useful for many

reasons. It would provide much information without requiring a large amount of blood sample, which is especially important in maternal and paediatric studies. If a trace element profile could be easily obtained, the trace element interactions in a wider range of diseases could be determined Also, unsuspected elemental deficiencies or overload could easily be detected during routine testing. Simultaneous multielement analysis also reduces the possibility of contamination from the handling of the sample which is required in sequential elemental analysis [39].

Other Biological Fluids

Whole blood is one of the most complex matrices of biological fluids. Both serum and plasma are constituents of whole blood, so the CMP-AES system should perform as well or better for these fluids. In some cases, the levels of elements in serum or plasma is a better indicator of health than the measurement of the levels in whole blood. Other biological fluids could also be analyzed.

Plasma

Blood plasma is the portion of blood remaining after the red and white blood cells and the platelets are removed. Blood plasma serves as the transport medium that delivers nutrients to the cells. It also removes the metabolites of

the cells and is involved in the regulation of physiological processes [4].

Serum

Serum is different from plasma in that it contains no fibrinogen. The absence of fibrinogen gives serum the advantage over plasma that no anticoagulants are needed, reducing the chance of contaminating the sample. However, the serum needs to be obtained by centrifugation, and not as much can be collected for a given amount of blood [4]. The measurement of iron in serum is more useful than the measurement of iron in whole blood. The levels of iron in whole blood does not provide adequate information on iron status because the amount of iron it its various forms may vary independently. The level of iron in serum, however, correlates well with serum transferrin which is a better indicator of iron status [56].

Urine and spinal fluid

The analysis of urine for primary and trace elements is important to determine how the body loses important nutrients. It can also be used as an indication of a recent occupational exposure, particularly for elements such as arsenic, chromium, lead, mercury, and nickel [37]. A decrease in the urinary excretion of an element can indicate a deficiency of that element [47]. The levels of elements in spinal fluid can be indicative of various diseases [37].

Miniaturize System

The electronics of the CMP-AES are capable of being reduced drastically in size. The microwave plasma portion of the setup should be able to be reduced to the size of a small microwave oven. Currently, microwave ovens consist of very similar parts: programmable power supply (high/medium/low), magnetron, and waveguide. To fully miniaturize the system, a method of detection consisting of smaller components would also have to be found.

Acousto-optic tunable filter

An acousto-optic tunable filter (AOTF) is a compact, tunable, narrow-band light filter [155]. An AOTF is constructed by bonding a piezoelectric transducer to a birefringent crystal, usually quartz or paratellurite (TeO_2). Acoustic waves are propagated through the crystal by applying a radio frequency (rf) signal to the transducer. The acoustic waves produce grating in the crystal that can diffract select wavelengths of the incident beam. The position of the bandpass can be controlled electronically over a wide spectral range by changing the frequency of the rf signal [155]. AOTF's have been used in fluorescence, emission, and spectroscopic imaging experiments [155-164] and to tune and stabilize a laser beam [165-166].

Horlick and Fulton have investigated using an AOTF for atomic spectrometry [156]. An inductively coupled plasma, a

glow discharge, and a hollow cathode lamp were used as atomic emission sources. By using two AOTF crystals, the wavelength range of 350-600 nm was covered. In one second, the entire spectrum was scanned with a resolution of 0.22 nm at 361 nm. Using an AOTF with the CMP-AES instead of the currently used spectrometer could help make it more suitable as a screening or clinical technique. The array type detectors would be unnecessary and a photomultiplier tube (PMT) or a photodiode could be used for the measurement of the analytical signal. This would greatly reduce the size and expense of the CCMP-AES.

Miniature detector

Miniature fiber optic spectrometers are commercially available for under $3000 (S2000 Fiber Optic Spectrometer, Ocean Optics, Inc., Dunedin, FL). One spectrometer is so small that it can fit on a computer card inside a notebook computer. Currently, the sensitivity and resolution may not be sufficient to analyze elements in blood in the low ppb range, but it might be able to measure the emission signal at the ppm level. If this system could be used with a reduced sized microwave plasma setup, the system would be portable to some extent. It would also be quite inexpensive for an atomic emission instrument.

Atomization Method for Other Techniques

The filament supported microwave plasma is a very efficient atomization source. The analytical volume is very compact and could be used as an atomization source for other analytical method. Laser excited atomic fluorescence spectrometry (LEAFS) using a both a flame [167] and a graphite furnace [168] has been used as a method to determine the levels of lead in blood. A detection limit of 4 ppb was determined using a flame and 10 ppt using a graphite furnace. As a source for atomic fluorescence, the plasma would not have to excite the sample, only atomize and excite it. This would allow a lower power to be used so that the analyte would have a longer residence time in the plasma and would be more completely dissociated [17]. The excellent performance of the CMP and the advantages it has over a flame and a graphite furnace might be able to lower the detection limits and improve the accuracy for LEAFS.

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BIOGRAPHICAL SKETCH

Arthur David Besteman was born in Grand Rapids, Michigan, on January 12, 1971. The son of Rev. Arthur and Audrey (Honderd) Besteman, Arthur has two older sisters, Debra and Diane. From the age of one to fifteen, Arthur grew up in Zeeland, Michigan. He moved to Wyoming, Michigan, where he attended Calvin Christian High School and graduated in June of 1989. He then enrolled at Calvin College and received his Bachelor of Science degree in May of 1993 majoring in chemistry. While at Calvin College, Arthur worked with Drs. Mark and Karen Muyskens, two physical chemists, studying the multiphoton ionization and fragmentation of methyl aniline. Arthur entered the graduate program at the University of Florida in August of 1993 and became a member of Dr. James Winefordner's research group, pursuing his Ph.D. in analytical chemistry.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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